ASSESSMENT OF ORGANOCHLORINE PESTICIDES AND ATRAZINE RESIDUES IN MAIZE PRODUCED IN GHANA USING GC-ECD/ GC-MS

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DECLARATION

This thesis is the result of research work undertaken by Elvis Djam Miensah towards the award of the Master of Philosophy degree in Nuclear and Environmental Protection in the Department of Nuclear Sciences and Applications, University of Ghana, under the supervision of Dr. J. R. Fianko and Dr. Sam Adu-Kumi.

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DEDICATION

This work is dedicated to my beloved parents Mr. Reuben Kumah and Mrs. Florence Kumah.

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LIST OF ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Diseases Registry
ATZ	Atrazine
BHC	Benzene hexachloride
CAS	Chemical Abstracts Service
CDC	Centers for Disease Control and Prevention
CIPAC	Collaborative International Pesticides Analytical Council
CSIR	Council for Scientific and Research Institute
DACT	Diaminochlorotriazine
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethene
DDT	Dichlorodiphenyltrichloroethane
DEA	Desethylatrazine
DHS	Disodium Hydrogencitrate Sesquihydrate
DIA	Desisopropyl
DIDEA	Didealkylated Atrazine
DOT	Department of Transport, United States
ECD	Electron Capture Detector
ECHA	European Chemicals Agency
EED	Estimated Exposure Dose
EPA	Environmental Protection Agency
EU-MRL	European Union Maximum Residue Limit
FAO	Food and Agricultural Organization

GC	Gas Chromatography
GHS	Globally Harmonised System
GSH	Glutathione
HA	Hydroxyatrazine
HCH	Hexachlorocyclohexane
HIATZ	1-Hydroxyisopropylatrazine
HLM	Human Liver Microsomes
HSDB	Hazardous Substances Data Base
IAEA	International Atomic Energy Agency
ILO	International Labour Organization
IMO	International Maritime Organization
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
ISO	International Standards Organization
IUPAC	International Union of Pure and Applied Chemistry
LH	Leutenizing hormone
LOD	Limit of Detection
MOFA	Ministry of Food and Agriculture
MRL	Maximun residue limit
MS	Mass Spectrometry
NIOSH	National Institute for Occupational Safety and Health -
NIP	National Implementation Plan
NIST	National Institute of Standard and Technology

NOES	National occupational exposure survey
NPAS	Northern Presbyterian Agricultural Services
OCPs	Organochlorine Pesticides
OECD	Organization for Economic Co-operation and Development
OEHHA	Office of Environmental Health Hazard Assessment
PCBs	Polychlorinated Biphenyls
PCCH	Pentachlorocyclohexane
POPRC	POPs Review Committee
POPs	Persistent Organic Pollutants
RTECS	Registry of Toxic Effects of Chemical Substances
SPE	Solid Phase Extraction
TCB	Trichlorobenzene
TSCD	Trisodium Citrate Dihydrate
UN	United Nations
UNEP	United Nations Environmental Program
USA	United States of America

ABSTRACT

Maize is consumed by over 95% of the Ghanaian populace and the practice of using atrazine, lindane and other organochlorine pesticides in its production has raised concerns about potential adverse effects on human health and the environment. A field survey was conducted in this study to assess farmers' knowledge of safe handling and use of these chemicals as well as toxicity awareness and symptoms among farmers and traders. Residues of the pesticides in maize samples as well as the potential health risk associated with exposure to them were also evaluated. Data obtained from the field survey indicate that a large proportion of farmers are at risk of pesticide poisoning through occupational exposure. Over 80 % of applicators do not apply safety precautions during pesticide mixing and application; resulting in considerable prevalence of pesticide related illnesses in the study area. Residues of lindane and atrazine were below detection limits of 0.005 mg/kg and 0.010 mg/kg respectively of the GC-ECD/MS used for the analysis; except in the Ashanti region where atrazine was 0.05 mg/kg which is within the EU-MRL of 0.1 mg/kg. However, minute concentrations of other organochlorines (endrin- 0.007 mg/kg; heptachlor- 0.023 mg/kg; alpha-endosulfan- 0.013 mg/kg) were found in maize samples from Brong Ahafo, Eastern, Central and Upper west regions. Though endrin and alpha-endosulfan were below the EU-MRL of 0.01 mg/kg and 0.10 mg/kg respectively, heptachlor was slightly above the EU-MRL of 0.01 mg/kg. The estimated doses for all these other organochlorines pesticides do not pose direct hazard to human health because their estimated dose values were lower than toxic thresholds and reference doses. However, due to the unsafe application practices by applicators, there exists a potential risk for systemic and carcinogenic health effects by these chemicals in the study are

CHAPTER ONE

INTRODUCTION

1.1 Background

The importance of maize (*Zea Mays*) in global food production cannot be overemphasized. Its cultivation is done by both commercial and peasant farmers (Onwueme and Sinha, 1991) as it is ranked third most widely cultivated and consumed cereal falling behind rice and wheat (Yayock et al, 1988). It is used significantly as a basic element of animal feed; raw material for industry as well as a significant source of nutrients for man. In Ghana, it is a major staple food crop whose domestic demand is projected to increase at an annual rate of 2.6% (MOFA, 2011). Though produced predominantly by smallholder farmers under rain-fed conditions, production has increased steadily since 1965 (FAO, 2008; Morris et al, 1999; SARI, 1996). Increasing population has also led to an increase in demand for maize and food from maize sources hence the extensive use of pesticides especially atrazine in its cultivation. It is estimated about 95% of the Ghanaian populace consumes food from maize sources (MOFA, 2001) and about 75% of the total maize produced in Ghana is also used as food while the remaining find their way into poultry feed and food grain sales industries (Muhammad, 1979).

The extensive use of pesticides to control pests on farm animals has been in practice in Ghana for over 40 years (Ntow *et al.*, 2005). Pesticides help improve crop yield, protect crop quality and reliability as well as reducing the cost of production. However, it has been established that pesticides could become detrimental to health if misused. One of the negative consequences of industrial development in the world has been the consequence of pesticide residues that are potentially hazardous to human health. Some of the negative effects of pesticides misuse include destruction of soil micro fauna and flora and undesirable residue accumulation in food crops (Edwards, 1986). The use of these chemicals over the years has led to the contamination of various environmental compartments such as soil, ground and surface water and air as well as numerous agricultural food products (Fianko et al, 2011).

Organochlorine pesticides (OCPs) are among the major group of pesticides used worldwide. They are synthetic organic insecticides that contain carbon, chlorine, hydrogen and sometimes oxygen. They are also called "chlorinated hydrocarbons" (Yeboah, 2009). Organochlorine pesticides (OCPs) constitute about 35% of pesticides used in organized farms and kraals in Ghana. (Awumbila and Bokuma, 1994). They have caused problems due to their lipophilic nature, persistence in the environment and potential toxicity. Historically, organochlorine pesticides (OCPs) had widespread applications. Sources of OCPs are mainly derived from agricultural applications (Wurll and Obbard, 2005). Some examples are lindane, endrin, heptachlor, endosulfan, dieldrin, p'p-DDE, p'p-DDT, p'p-DDD, gamma-Chlordane, methoxychlor, delta-HCH, β-HCH, endosulfan sulphate and aldrin.

Lindane (gamma-hexachlorocyclohexane) (γ-HCH), gammaxene or Gammallin is an organochlorine (OCP) chemical and the only active isomer of hexachlorocyclohexane (Maryadele and O'Neil, 2001**.** It has been used over the years as both agricultural and pharmaceutical chemical. Like all OCPs, when released into the environment, it remains intact for exceptionally long periods, becomes widely distributed throughout the environment, accumulates in tissues of living organisms and toxic to both humans and animals (Stockholm convention, 2004). So toxic are OCPs that their importation, sale and use in Ghana has been banned since 2002 (Ghana NIP, 2007). Lindane has been used over the years in maize production to control pests. Lindane has also been used in Ghana on cocoa farms, vegetable farms and for control of stem borers in maize (Ntow et al, 2006).

Lindane belongs to the class IIB group of insecticides; because it is moderately acutely toxic. The international trade of lindane is restricted under the prio- informed consent of the Rotterdam Convention (UNEP, ILO, WHO, 1994). Under the Stockholm Convention, the agricultural use as well as production of lindane was banned due to its toxicity (Stockholm Convention, 2009). Moreover it was classified in 1987 by International Agency for Research on Cancer (IARC) as a group 2B chemical; meaning it is a possible human carcinogen (IARC, 1987). The principal areas lindane affects in humans are the nervous system, liver and kidneys. A range of symptoms such as convulsions, headache, seizures and dizziness occur when exposed to large amounts of lindane (U.S. FDA, 2003; Alvarez-Pedrerol, 2008).

Unlike lindane, atrazine is a herbicide. The worldwide increase in maize and other cereal production could be attributed to the use of herbicides. Works that reveal agricultural loses of about 500 percent without herbicide use only highlight the significance of these herbicides in modern management of weeds in the production of maize (Bridges, 1994). Herbicide application is thus an efficient way to check weed infestation; helping to achieve speedy breakthroughs in increasing maize production. This is further highlighted by several works done over the years. A research revealed positive maize yields upon atrazine application (Miller and Libby, 1999). In a similar work, it was reported that maize gave higher yields when weed management was done with herbicides as opposed to controlling weeds culturally (Becker and Staniforth 1981). It has also been reported that application of herbicides gave 65-90% weed control and 100-150% more maize yield (Jehangeri et al, 1984). Atrazine is a widely used herbicide as it is the second largest selling pesticide in the world (was largest till 2001) (Cox, 2001. In Ghana, atrazine is the major herbicide used. It is a ubiquitous, persistent and highly mobile pesticide used primarily on maize. Atrazine highly interferes with the photosynthetic pathway of plants; inhibiting photosynthesis (MAFF, 1993).

In spite of its use in increasing maize yield through weed control, atrazine has been found to reduce the ability to farm animals to successfully reproduce (Cox, 2001). It induces estrogen production while inhibiting production of testosterone; this upsets the balance between these two hormones causing chemical castration (Hayes et. al, 2002). Research has shown that, the level of pesticides in food in many developing countries including Ghana is escalating (Ntow, 2001). Most of the levels found in humans are related to current and past use of these chemicals. It is not surprising therefore that residue of chlorinated pesticides in food have raised major concerns. Earlier studies in Ghana indicated the presence of pesticide residues in meat, water, sediment, vegetables, fruits, fish and soil at locations unknown for pesticide usage (Osafo and Frempong, 1998; Aboagye, 2002; Mensah *et al.*, 2004; Amoah *et al.*, 2006; Adu-kumi et al, 2010). The high application of pesticides has resulted in food contaminations (Wilson *et al.*, 2007); ingestion of which has been linked with health conditions. Contaminated foods are a major source of morbidity, mortality and increased risk of skin cancer, destruction of neurological cellular functions, chronic neurotoxicity, bladder and lung cancer even at very low concentrations (Mensah *et al.*, 2004; Sun *et al.*, 2006). The U.S FDA in a 2003 report said that most of the adverse human health effects by lindane reported have been related to agricultural uses (U.S. FDA, 2003). The high consumption of maize by Ghanaians has thus warranted the need to assess the levels of atrazine and lindane residues so as to correctly estimate human dietary exposure and also refine risk assessments.

1.2 Statement of problem

Maize is the most important cereal crop produced in Ghana and it is also the most widely consumed staple food in Ghana with increasing production since 1965 (FAO, 2008; Morris et al., 1999).

Atrazine, lindane and other organochlorine pesticides (endrin, heptachlor, endosulfan, dieldrin, p'p-DDE, p'p-DDT, p'p-DDD, gamma-Chlordane, methoxychlor, delta-HCH, β-HCH, endosulfan sulphate and aldrin) have been used extensively in maize production; however, there is no routine monitoring of this food item to check the residue level of organochlorines such as lindane and atrazine even though they are toxic. Monitoring of pesticide residues in export crops such as cocoa is routine and stringent due to high standards and strict monitoring programmes set by buyers but when it comes to domestically consumed food, no such stringency exists. Though lindane use was banned in Ghana in 2009 (EPA, 2013) and atrazine (banned in EU) for their toxicity, they continue to be available on the local market and being used by farmers and traders. These pesticides have been identified to affect the nervous system, liver, kidneys, cause chemical castration and are possible carcinogens. However, farmers continue to use lindane, atrazine and other organochlorine pesticides without protective measures and without knowing what the possible health implications of these chemicals are. Unfortunately, their continuous use means they may be present in many staple foods in Ghana; continuous consumption of which could have serious health implications. There is also the concern that the huge number of consumers of food from maize sources in Ghana may be bio-accumulating lindane, atrazine and other organochlorine pesticide residues with long term health effects.

1.3 Objectives of the study

The objectives of this study are:

- To assess the knowledge, practice and attitudes regarding safe use of atrazine lindane and other organochlorine pesticides, toxicity awareness and symptoms among farmers and traders

- To determine the levels and distribution of atrazine, lindane, endrin, heptachlor, endosulfan, dieldrin, p’p-DDE, p’p-DDT, p’p-DDD, gamma-Chlordane, methoxychlor, delta-HCH, β-HCH, endosulfan sulphate and aldrin in maize in Ghana.

- To evaluate the potential human health risk associated with lindane, endrin, heptachlor, endosulfan, dieldrin, p’p-DDE, p’p-DDT, p’p-DDD, gamma-Chlordane, methoxychlor, delta-HCH, β-HCH, endosulfan sulphate, aldrin and atrazine ingestion through maize in Ghana.

1.4 Relevance and Justification

Atrazine and organochlorines (lindane, endrin, heptachlor, endosulfan, dieldrin, p’p-DDE, p’p-DDT, p’p-DDD, gamma-Chlordane, methoxychlor, delta-HCH, β-HCH, endosulfan sulphate and aldrin) are pesticides that are of great importance in the agriculture and the health sectors. However, they have been identified to affect the nervous system, liver, kidneys, and are possible carcinogens. They are extensively distributed in the environment due to their persistence and resistance to degradation. They bio-accumulate in human tissues and this eventually poses serious health risks to humans and animals (Ejoba *et al*., 1996). Maize occupies a very special position in the Ghanaian diet hence the need to know the residue level of these chemicals in maize so as to protect the health of farmers, traders and consumers. This work would provide relevant data for establishing a monitoring programme for organochlorines especially lindane and atrazine in maize grown in Ghana. This would enable authorities to check for compliance with Ghanaian legislation on lindane and atrazine. It would also provide relevant data to estimate the amount of these chemicals consumed in our diet. This work would also provide relevant data for the Ministry of Food and Agriculture (MOFA-Ghana), Ghana Standards Authority, Food and Drugs Authority, Ghana EPA, Joint meeting on pesticide residues (JMPR) of the UN. In addition, the level of farmers’ and traders’ knowledge, practice and attitudes regarding safe use of lindane and atrazine, toxicity awareness and symptoms would be known in order to be used in making informed policy decisions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Maize (*Zea mays)* belongs to the grass family *Graminaceae*. As a result of its significance among cereal crops, it is cultivated worldwide (IITA, 1991). Its cultivation is done by both peasant and commercial farmers' worldwide (Onwueme and Sinha, 1991). Worldwide, it is ranked third in terms of production and consumption, following rice and wheat (Yayock *et al,* 1988). Maize is a significant source of nutrient for man, a basic element of animal feed and raw material for industry (Romains, 2001). Some industrial products of maize are corn syrup, corn starch, corn flakes, corn oil and maltodextrins. It also serves as a significant source of nutrients such as iron, protein, vitamin B, carbohydrates as well as serving as biofuel (Romains, 2001). Maize is used largely as livestock feed and raw material for industrial products in highly industrialized countries. In developing countries however, it is mostly used as food for consumption (IITA, 2007).

In Ghana, it is the most grown cereal (Gounou *et al.*, 1994; Yayock *et al,* 1988). Its cultivation is done nationwide in all the ecological zones. It is the most important cereal crop produced in Ghana and it is also the most widely consumed staple food in Ghana with increasing production since 1965 (FAO, 2008; Morris *et al*., 1999). In Ghana, maize is produced predominantly by smallholder resource poor farmers under rain-fed conditions (SARI, 1996). Some varieties of maize grown across the various ecological zones in Ghana are Okomasa, Abeleehi, Obatanpa, Dadaba, Mamaba, CIDA-ba and Golden Jubilee Maize.

2.2 Maize Production in Ghana

Maize accounts for 55 percent of grains produced (FAO STAT, 2012). It is followed closely by paddy rice, sorghum and millet. The average yield of maize in Ghana was estimated to be 1.9 Mt/ha in 2010 (MOFA, 2010). Maize production over the period 1990-2010 shows significant increases starting from year 2008. It was however not possible to explain to what extent the production increase was due to fertilizer subsidies in 2008, favourable rains and high food prices which could have stimulated domestic production over the period 2008-2010. Though Maize is grown throughout Ghana, the leading producing areas are mainly in the middle-southern part (Brong Ahafo, Eastern and Ashanti regions) where 84 percent of the maize is grown, with the remaining 16 percent being grown in the central and northern regions of the country (Northern, Upper East and Upper West provinces).

2.3 Marketing and Trade of Maize in Ghana

Women engaged in maize trading, known as “market queens”, dominate the local and regional maize markets in Ghana. Techiman in the Brong Ahafo region is a major feeder market in Ghana because of the maize coming from the main producing areas in Brong-Ahafo. Local wholesalers acquire their maize from farmers and then sell to long-distance traders serving urban markets throughout the country (FAO, 2006). From Techiman the maize is then directed to Accra and/or Bolgatanga, at the border with Burkina Faso, and/or Cote d’Ivoire. Tamale in the Northern region is another important feeder market for maize in Ghana.

2.4 Maize Consumption in Ghana

Though maize supply in Ghana has been increasing steadily over the past few years with an average supply at 1.4 million MT over the period 2005-2010, human consumption is competing with the poultry and livestock industry. While there is no reliable data for maize used in animal feed, the Government of Ghana estimates that 85% of Ghanaian maize is for human consumption; the remaining 15 percent is used mainly poultry feed sector. In a 2008 survey from major feed mills in Ghana, about 250 000 MT of maize is used in the poultry feed sector annually (WABS consulting Ltd. 2008). This is in line with the data on consumption of maize in 2006 where the poultry industry absorbed 170 000 MT of domestic production. In the northern region of Ghana, millet and sorghum are the main cereals produced and consumed, but in times of scarcity maize, which is usually a surplus crop, is used as a substitute for these grains. White maize consumption is projected to increase due to population growth and increasing per capita income (MOFA, 2011). The high consumption of maize by Ghanaians has thus warranted the need to know the levels of these pesticide residues so as to correctly estimate human dietary exposure and also perform risk assessments. For instance, an estimated 95% of the Ghanaian populace consumes food from maize, such as “Akple, “Kpekple”, “Banku”, ”Kenkey”, ”Apreprensa”, Porridge etc (MOFA , 2001). It is also estimated that 75% of the total production of maize is used as food by farm-holds or communities and the remaining find their way into starch manufacturing industry, poultry feed and food grain sales (Muhammad, 1979).

2.5 Pesticides

Food and Agricultural Organization has defined pesticide as any substance or mixture of substances intended for preventing, destroying or controlling any pest; including vectors of human or animal disease, unwanted species of plants or animals causing harm during production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for control of insects, arachnids or other pests in or on their bodies (FAO, 2002).

Pesticides have produced enormous benefits such as controlling pests and diseases, eradicating weeds and pests on farm animals, preserving crops from insect attacks, increasing food quality as well as reducing cost of production and hence price of produce (Hayes and Laws, 1990). Their effective use in agriculture can be traced to as far as 1763. To the farmer, pesticides provide benefits through prevention of yield losses, improved crop quality, enhanced market opportunities, facilitation of farm work and harvest and improving cost/profit ratio. The consumer also benefits through abundant food supply, safe from pest, available throughout the year and promotes good health. Notwithstanding these benefits, their extensive and unregulated application has raised concerns with regards to their health and environmental effects.

2.6 Classification of Pesticides

Pesticides are classified based on the following:

- Target pest which deals with the type of pest for which it is made to eliminate.
- Mode of action. This type of pesticide classification is based on how the pesticides affect their respective pests.
- Chemical nature: Focuses on the chemicals that make up the pesticide.
- Use: this focuses on the specific use or area of application of the pesticides. For instance whether it is applied on the leaves, soil or selective between narrow leaves and broad leaves.

2.6.1 Classification Based on Target Pest

This classification is based on the type of pest the pesticide is intended to eradicate.

Table 2.1: Classification of pesticides by target pest

Pesticide	Example	Target pest
Insecticide	Lindane, Karate, Cymbush, Comfidor	Insects
Fungicide	Dithane M-45, Kocide	Fungi
Herbicide	Glyphosate, Gramoxone, Atrazine	Weeds
Rodenticide	Klerat	Rodents
Nematicide	Methyl-bromide or Diethyl-Dibromide	Nematodes
Miticide	Dimethoate	Mites
Bactericide	Streptomycin	Bacteria
Acaricide	Dimethoate	Mites, ticks and spiders
Repellent	N,N-Diethyl-meta-toluamide (DEET)	Keeps pests away
Growth regulators	Butralin, Fair, Royaltac M, Sucker-Plucker, Off-Shoot, Contact-85	Stops, speeds up or otherwise change normal plant processes
Predacides	sodium fluoroacetate (1080)	Vertebrate pests
Avicide	DRC 736, DRC 1327	Birds
Molluscicide	Phorate	Mollusks such as snails, slugs
Larvicide	Aquabac, Teknar, Vectobac, and LarvX	Larvae
Termiticide	Chlorpyrifos	Termites, ants
Piscicide	rotenone, saponins, TFM, niclosamide	Fish
Adulticide	malathion, naled, chlorpyrifos, permethrin, resmethrin, sumithrin,	Adult insects
Arboricide / silvicide	bipyridylium zwitterion, paraquat	Trees, bushes, shrubs
Ovicide	Isoxathion	Eggs

Source: http://westnile.ca.gov/special/category_a/?page=Chapter2.htm

2.6.2 Classification by Mode of Action

Pesticides can also be grouped according to how they work (their mode of action) as illustrated in table 2.2

Table 2.2: Classification by mode of action

Pesticide	Mode of action
Antifeedant	inhibits feeding while insects remain on the treated plant; the insects eventually starve to death
Anti-transpirant	reduces transpiration
Attractant	lures pests to treated locations, e.g. sex attractant
Feeding stimulant	causes insects to feed more vigorously
Semiochemical	Pheromones, allomones and kairomones; substances emitted by plants or animals, which stimulate or inhibit certain behavioral activities of insects
Synergist	enhances the effectiveness of an active agent
Contact	Kills pests on contact
Stomach poisons	Kills pests when swallowed. With insects pest, these enter insect's body during feeding
Systemic	Taken into the blood of an animal or sap of plants. They kill insects without harming host. For instance, systemic pesticides applied to leaves, stems, or roots of plants move through the plant to other parts of the plant. An insect pest acquires translocated pesticides during feeding.
Fumigants	These are gases, which kill when they are inhaled or otherwise, absorbed by pest.
Pheromones	Affects pests by changing their behaviour.
Sterilants	Makes pests unable to produce
Desiccant Dusts	They cause loss of body fluids and death by dehydration. Common desiccants include boric acid.
Insect growth regulators	These chemicals affect the ability of insects to grow and mature normally. They usually mimic growth hormones.
Protectant	Applied to plants, animals and structures to prevent entry or damage by pests.
Chemosterilant	They cause sexual sterility in either female or male insect or animal.

Source: http://westnile.ca.gov/special/category_a/?page=Chapter2.htm

2.6.3 Classification by Chemical Nature

Pesticides can be classified into two main chemical groups and these are organic and inorganic (Koehler and Belmont, 1998).

2.6.3.1 Inorganic

These are made from compounds that do not contain carbon. They are generally crystalline and salt-like in appearance, and soluble in water, e.g. Copper hydroxide, $Cu(OH)_2$. They were widely used to control insect pests before the discovery of synthetic organic compounds such as dichlorodiphenyltrichloroethane (DDT). They are not fat soluble and act only as stomach poisons. Examples are Calcium arsenate, Lead arsenate, Paris green (Copper acetate + Copper arsenate) (Koehler and Belmont, 1998).

2.6.3.2 Organic

Organic pesticides are made from compounds containing carbon in addition to hydrogen. They can also contain oxygen, sulphur, phosphorus, chlorine and nitrogen in the molecule. Organic compounds constitute the majority of all modern pesticides. Most are insoluble in water. Organic pesticides can be further subdivided into two groups:

- SYNTHETIC organics & NATURAL organics (Koehler and Belmont, 1998)

SYNTHETIC ORGANICS

The synthetic organics can further be subdivided into several major groups, including the following:

- **ORGANOCHLORINES** (sometimes called chlorinated hydrocarbons): These are organic compounds containing one or more chlorine atoms as hydrogen substitutes in their molecule. Until recently, the organochlorines were the most commonly used pesticide. Some have been banned or are severely restricted; an example is Lindane

- **ORGANOPHOSPHATES**: These are the next major group of synthetic organic pesticides. Chemicals in this group contain phosphorus bonded to a carbon atom in the molecule. From safety point of view, the organophosphates are known to cause most pesticide related illnesses in humans.

- **CARBAMATES**: These chemicals contain nitrogen as a C – N bond in the molecule. Like the organophosphates they also cause poisoning in humans. They are derived from carbamic acid and act as stomach poisons and to a lesser extent, residual contact pesticides, on a fairly broad spectrum of plant pests. Some are effective systemically on insects, mites and nematodes (Casida and Quistad, 1998; Peter and Cherian, 2000).

NATURAL ORGANIC PESTICIDES

Originally, these pesticides were obtained from plants. Included in these groups are pyrethrums extracted from chrysanthemums. Certain organics are now prepared synthetically and are commercially available. Pyrethroids are synthetically produced compounds that mimic the structure of naturally occurring pyrethrums.

- **MICROBIAL PESTICIDES**: The active substances in this type of pesticides are fungus, protozoa, bacteria and virus. One such microbial pesticide is *Bacillus thuringiensis*.

2.6.4 Classification by Use

Herbicides, being one of the most widely used pesticides, may be conveniently classified by use that is whether they are applied to the soil or foliage, and by their selectivity or how they affect target plants (Table 2.3)

Table 2.3: Classification of pesticides by use

PESTICIDE	USE
Selective pesticides	These are used to control weeds without damaging desirable plants. For instance, narrow-leave weeds can be preferentially controlled. Cognisance should be taken of the fact that selectivity is only relative, and misuse can result in damage to desirable plants.
Foliage-applied	These are applied on leaves & other above ground parts of plant. These serve as contact killing only the parts of the plant actually sprayed. Complete coverage of weeds is a must for complete kill.
Soil-applied selective	These are applied to the ground and are recommended for pre-plant, pre-emergence, post-emergence treatments.
Non-selective	Used when complete removal of vegetation is desired. They kill most plants when applied

Source: http://westnile.ca.gov/special/category_a/?page=Chapter2.htm

Moreover, the World Health Organization (WHO) now uses the Acute Toxicity Hazard Categories from the Globally Harmonized System (GHS) as the starting point for classification.

Table 2.4: Classification of pesticides based on WHO standards

WHO Class	Description	LD_{50} for the rat (mg/kg bodyweight)	
		Oral	Dermal
Ia	Extremely Hazardous	<5	<50
Ib	Highly Hazardous	5-50	50-200
II	Moderately hazardous	50-2000	200-2000
III	Slightly Hazardous	Over 2000	Over 2000
U	Unlikely to present acute hazard	5000 or higher	5000 or higher

Source: WHO, 1998

2.7 Pesticide Formulations

Pesticides are rarely used in their pure form (technical grade). They are processed into a usable form for direct application. The active ingredients in a pesticide product are the chemicals that control the target pest. A typical pesticide product also has other ingredients called ‘inert’ (or inactive) ingredients. These are added to dilute the pesticide or render it safer, more efficient, easier to measure, mix and apply, and more convenient to handle. The process of preparing mixtures of active ingredients with these other materials is known as formulation. This formulation is the final physical condition in which the pesticide is sold for use. The product from the process of formulation is known as the formulation. It is the formulated product that we put in our spray tanks and mix with water for use. The following types of formulations are commonly in use:

- Dry formulations examples are dusts, granules and baits

- Liquid formulation examples are wettable powders, emulsifiable concentrates and flowables

- Gaseous formulations such as aerosols

2.8 Metabolites

Inert ingredients and active ingredients make up pesticide formulations. The inert ingredients are usually considered proprietary information while the active ingredient component of a pesticide formulation is responsible for its toxic effects. However, the active ingredients of some pesticide formulations are converted to metabolites (Cloyd and Bethke, 2011). A metabolite is a compound formed during metabolism (an overall set of chemical reactions that occur in an organism or cell). Pesticides that are released into the environment either decrease or increase in level of concentrations as the parent chemicals are converted into metabolites. Some metabolites have equal or greater toxicity than the parent chemical while some are less toxic than the parent compounds (Vanloon and Duffy, 2000). Generally, the metabolites of pesticides are more stable and possess greater degree of polarity than the parent chemical. Their molecular weights are lower and the compounds are more completely oxidized. These disparities make the metabolites more water soluble, thus, increasing mobility and the likelihood of transport (Barrett, 1996; Kolpin *et al.,* 1996; Mangiapan *et al.,* 1997; Kalkhoff *et al.,* 1998).

2.9 Pesticide Residue

Pesticide residue refers to the amount of a pesticide or ingredients in the mixture found in or on a raw agricultural commodity or in a processed food. The definition also includes residue of degradation products of the pesticide; whether those products are the result of plant metabolism or some other degrading process. Thus the residue of concern may be the parent compound, a metabolite of the parent compound or both.

2.10 Pesticide Tolerance/Maximum Residue Levels (MRLs)

This is the amount of pesticide residue legally allowed by regulatory bodies to remain in or on a harvested crop. Public concern for food safety and security has led to maximum residue levels (MRLs) which ensure that consumer exposures to harmful pesticides are restricted. Pesticide usage is thus restricted to registered standards to ensure the circulation of only food produce that have been treated with approved pesticides and comply with the approved MRLs (Chan, 2000). Exposure of consumers or the general population to these residues most commonly occurs through consumption of treated food sources, or being in close contact to areas treated with these pesticides such as farms, lawns and around houses (USEPA, 2005).

2.11 Importation and use Of Pesticides In Ghana

The extensive use of pesticides in Ghana over the years has resulted in an increase in their demand and hence their importation from other countries. These importations have become necessary simply because; the formulation of some of the pesticides cannot be done in Ghana.

Table 2.5: Quantities of Pesticides Imported into Ghana during the period 2008 – 2012

Year	**2008**	**2009**	**2011**	**2012**
Herbicides (Solid Mt)	1,430	1,000	854,400	991,300
Herbicides (Liquid '000Lt)	6,100	8,980	30,300	14600
Insecticides (Solid Mt)	280	60	832,810	543,000
Insecticides (Liquid '000Lt)	3,270	3,400	4,800	4,210
Fungicides (Solid Mt)	1,560	340	596,000	180,000
Fungicides (Liquid '000Lt)	180	950	52,000	186,000

Source: EPA, GHANA, 2013.

In Ghana, the use of pesticides has been employed in agriculture not only to control and eradicate pests but also in the public health sector for disease control. However, pesticide usage has increased rapidly in quantity over the past ten years (Hodgson, 2003). Pesticides are used predominantly in cereals, cocoa, legumes, coffee, vegetables, fruits, tuber crops, sugarcane and oil palm production. Most farmers in animal husbandry also use pesticides very often in the rearing of the animals. In Ghana, there are different kinds of pesticides that are found on the market which include; organophosphate, carbamate, organochlorine and pyrethroids, chiefly among them being the organochlorine pesticides. The use of organochlorine pesticides began in Ghana around 1948, with the introduction of DDT in the form of emulsion spray in the cocoa industry (EPA Ghana, 2008).

The extensive use of the organochlorines can be attributed to the fact that they are cost effective and used for a wide range of activities. In response to the global ban on organochlorine pesticides due to environmental persistence, bioaccumulation and biomagnifications along the food chain (Moshi *et al.,* 1980). Ghana has also banned the use of organochlorine pesticides such as aldrin, endrin, dieldrin and DDT (EPA Ghana, 2008). However, some Ghanaian farmers still use these organochlorine pesticides such as endosulfan and lindane due to their low cost and efficacy. For instance, in a study of 30 farms and 110 kraals in Ghana, 20 different pesticides were found to be in use; with lindane being the most widely distributed and used representing 35% of those applied on farms (Fianko et al, 2011). Hand dressing is the most suitable method for pesticides application. Majority of the pesticides are employed in farming regions such as Eastern, Ashanti, Western and Brong Ahafo (Amoah *et al*., 2006).

2.12 Pesticide Management and Control Policies in Ghana

The Pesticide Control and Management Act of Ghana (Act 528) was enacted in 1996 to ensure effective control and management of pesticides. The law requires the registration of all distributors of agrochemicals and pesticides. Part Two of the EPA Act, 1994 (Act 490) - Pesticides Control and Management makes the Environmental Protection Agency of Ghana the lead agency responsible for a comprehensive pesticide regulatory program. In that capacity, the EPA has the sole authority and responsibility to register all pesticides imported, exported, manufactured, distributed, advertised, sold or used within Ghana. This allows the EPA to confirm that the product provides for both human and environmental protection. Act 528 of 1996 defines four classes of pesticides: general use (Class 1), restricted use (class two), suspended pesticide (class three) and banned pesticide (class four). Pesticides in classes two, three and four must be subjected to the Prior Informed Consent procedure. If the use of a pesticide results in adverse effects on humans, animals or the environment, it may be suspended or restricted (EPA Ghana 1994, 1997).

2.13 Pesticide Usage in Food Production

There are diverse views on pesticides usage in food production. These views often exhibit extreme differences; while some are very pro (in favour of pesticides), some are very anti (against pesticides). "Unless changes are made it may not be far from truth to say that, rather than feeding the hungry, pesticides will be poisoning the hungry to feed the well-fed (MacManus, 1988)". Moreover, Humanity in the 21st century can banish hunger, end nutritional deficits in its children, and save virtually all of the remaining wild-lands in the process. But there are only two ways to do it: either murder four billion people, or use chemicals and biotechnology to maintain and increase yields on land already under farming (Avery, 1995). The capacity to produce food is faced with numerous challenges. These include a projected world population increase of about 10 billion by 2050 and a declining arable land to population ratio. This population explosion is mostly in developing countries where according to the Food and Agricultural Organization, about 500 million people are already undernourished (Anon, 1990a).

Numerous examples can be made of the impact of pesticides on food production. For instance the Irish potato famine of the nineteenth century resulted in the death of millions. Mention can also be made of the 700,000 deaths from starvation as a result of the 20th century fungal destruction of the German potato crop (Anon, 1992b). Research works which estimate that food supplies would immediately fall to between 30 and 40% as a result of pest destruction underline the importance of pesticides to crop yield (Anon, 1990b; Anon, 1992a). The first recorded use of chemicals for pest control was 2500 BC. Chemical control however only gained prominence in the last 50 years (Hock et al, 1991). The earliest pesticides used were mostly inorganic products or derived from plants. Mention can be made of burning sulphur to control insects and mites; hellebore was used to control body lice while nicotine was also used to control aphids. Pyrethrin was however used to control numerous diverse kinds of insects (Hock et al., 1991). In addition to increasing crop yields, pesticides prolong the shelf life and viability of stored products and also significantly reduce post-harvest losses.

2.14 Pesticides and Health

The use of pesticides is now routine practice worldwide. However, many concerns have been raised about their safety especially when not used properly. Pesticides, if not properly used cause significant damage to human health, environment and wildlife. Humanity is now exposed to many different toxic compounds due to changes in lifestyle. The extensive use of chemicals over the years has led to the gradual contamination of food, water, the air we breathe and the environment in general and human exposure to such chemicals while even in the womb of the mother through a contaminated food chain is cause for concern (Lederman, 1996). Pesticides after application are widely distributed in environmental compartments, contaminating the air, soil and water (Verstraeten et al., 2002; Pucarević et al., 2003; Pucarevic and Sekulić, 2004; Pucarević et al., 2010). Fenik et al., (2011) argued that the widespread use of pesticides, their stability and tendency of bio-accumulation, make them particularly dangerous for man, hence, exposure through consumed food must be pointed out as a special risk health. Exposure to pesticides may cause either acute and/or chronic health effects in those who are exposed (US EPA, 2007). Exposure to pesticides can cause diverse adverse health effects ranging from skin and eye irritations to severe effects such as nervous system disruptions, reproductive complications and cancer. A 2007 systematic review which associated lymphoma and leukaemia to pesticide exposure concluded that cosmetic use of pesticides should be decreased (Bassil et al., 2007). Reference can also be made to other negative outcomes from pesticide exposure including neurological effects, birth defects, foetal death (Sabon et al., 2007) and neuro-developmental disorder (Jurewicz and Hanke, 2008). Estimates have been made of about 3 million agricultural workers in developing countries experiencing severe poisoning from pesticides with about eighteen million deaths (Miller, 2004).

A study was conducted to examine the extent to which pesticide-associated symptoms occur in some Ghanaian farmers involved in irrigation projects. It was revealed that about 36% of the farmers had experienced negative side effects such as headache, dizziness, fever, blurred vision, vomiting and nausea after applying pesticides (Clarke, 1997). This study also revealed direct linkages between the use of personal protective equipment and the extent of the negative side effects. Pesticides can enter the human body by inhalation (aerosols, dust and vapour), by ingestion of contaminated food and water (oral exposure) and by direct contact of pesticides with skin (dermal exposure). In developing countries like Ghana, pesticides exposure has become rampant especially during the application of insecticides in farm. This is because most farmers do not take good precautionary measures such as wearing personal protective clothing.

In the public health sector, pesticides usage has caused health defects such as cancers (Sabon et al., 2007). Mention can be made of studies on the effects of pesticide exposure on the risk of cancer especially leukaemia (Mansour, 2004). Pesticides are used excessively to the extent that some of the residues get into foodstuff. In November 2012, it was reported by the Daily Graphic newspaper that, the death of eight family members after eating a meal of beans at Shaibuipe in Northern Region of Ghana was attributed to acute poisoning of a pesticide (atrazine which was used as herbicide) (Daily Graphic, 24/11/12). It has been discovered in United States of America (USA) that, about 4,500 accidental poisonings occur each year resulting in about 50 deaths per year. Many more are believed to die of cancer caused by pesticides exposure. (Mckinney and Schoch, 2003).

2.15 Organochlorines

Organochlorine pesticides (OCPs) are among the major group of pesticides used worldwide. They are synthetic organic insecticides that contain carbon, chlorine, hydrogen and sometimes oxygen. They are also called "chlorinated hydrocarbons" (Yeboah, 2009). Organochlorine pesticides (OCPs) constitute about 35% of pesticides used in organized farms and kraals in Ghana. (Awumbila and Bokuma, 1994). They have caused problems due to their lipophilic nature, persistence in the environment and potential toxicity. Historically, organochlorine pesticides (OCPs) had widespread applications. Sources of OCPs are mainly derived from agricultural applications (Wurll and Obbard, 2005). Some examples are lindane, endrin, heptachlor, endosulfan, dieldrin, p'p-DDE, p'p-DDT, p'p-DDD, gamma-Chlordane, methoxychlor, delta-HCH, β-HCH, endosulfan sulphate and aldrin. There is empirical evidence of higher concentrations of OCPs residues in fatty foods including milk (Leng *et al.*, 2009). OCPs have been found in beef, milk, yoghourt, cheese, beef fat (Darko *et al.*, 2008). OCPs residues bioaccumulates in the fatty tissues of exposed animals and humans, and may be responsible for a wide variety of health effects.

2.16 Lindane

Lindane, the gamma isomer of hexachlorocyclohexane (HCH), is a broad spectrum insecticide. It was originally evaluated by the JMPR in 1966 (under the name gamma-BHC) and re-evaluated for residues several times up to 1989, and is included in the CCPR (Codex Committee on Pesticide Residues) periodic review programme. Lindane and its related isomers, alpha- and beta-HCH, have been listed under the Stockholm Convention by the Persistent Organic Pollutants Review Committee (POPRC). Lindane, alpha-HCH, and beta-HCH have been included under provisions of Annex A of the Convention. Although lindane is the only HCH isomer that exhibits insecticidal properties, for every ton of lindane that is manufactured, approximately nine tons of toxic and persistent mixed-isomer HCH wastes are produced (Commission for Environmental Cooperation of North America, 2006). Between 2 and 4.8 million tons of HCH wastes remain worldwide from lindane production (Vijgen, 2006). These stockpiles have been poorly managed in many countries, creating hazardous waste sites that contaminate local water sources, habitats, and communities. In addition, these wastes become a source of long-range contamination to remote Arctic ecosystems where HCH isomers are among the most prevalent and persistent contaminants. As of April 2009, Lindane (gamma-HCH) is banned for use in at least 52 countries, restricted or severely restricted in 33 countries, not registered in 10 countries, and registered in 17 countries (Centre for Environmental Cooperation of North America, 2006). The Production and agricultural use of Lindane is also banned in the 169 countries that are parties to the Stockholm Convention, though pharmaceutical use is allowed until 2015 (UNEP-POPS-COP, 2009).

2.16.1 Identity of Lindane

ISO common name: Lindane

Chemical name:

IUPAC: 1α, 2α, 3β, 4α, 5α, 6β-hexachlorocyclohexane

CA: 1α, 2α, 3β, 4α, 5α, 6β-hexachlorocyclohexene

CAS Registry No: 58-89-9

Molecular weight: 290.8 (290.9) (WHO, 1991)

CIPAC No: 488

Synonyms and trade names: hexachlorocyclohexane (gamma-isomer); gamma-HCH, gamma BHC (WHO, 1991)

Molecular formula: $C_6H_6Cl_6$

Structural formula: (WHO, 1991)

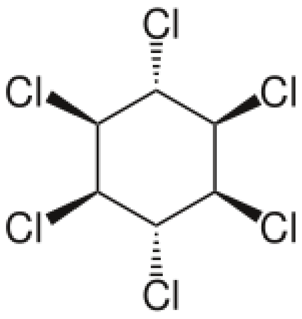


Figure 2.1: Structure of Lindane

The physical and chemical properties of Lindane are presented in Table 2.6

Table 2.6: Physical and chemical properties of lindane: Pure active ingredient and Technical material.

Property	Value	Reference
Minimum Purity	99.5%.	(WHO, 1991)
Appearance:	Colourless, crystalline solid.	(WHO, 1991)
Vapour pressure:	4.34 mPa (3.26x10-5 mm Hg) at 20°C.	(WHO, 1991)
Melting point:	112.8°C	(WHO, 1991)
Octanol/water partition coefficient:	3.2-3.7	(WHO, 1991)
Solubility:	10 mg/l in water at 20°C; 67 g/l in ethanol, slightly soluble in mineral oils, soluble in acetone, aromatic and chlorinated solvents.	(WHO, 1991)
Relative density:	1.85 g/cm^3, temperature not stated	
Hydrolysis:	• pH 5, stable, half-life 173.3 or 115.5 days respectively in 0.05 or 0.10 M buffer; 5% transformation after 30 days. • pH 7, stable, half-life 309.4 or 281.7 days respectively in 0.05 or 0.10 M buffer; 5% transformation after 30 days. • pH 9, unstable, half-life 36.3 or 35.4 days respectively in 0.05 or 0.10 M buffer; 43-44% transformation after 30 days.	(Mirfakhrae, 1986)
Photolysis:	• Study 1, resistant to natural sunlight in water, ≥90% recovery after 28 days.	(Norris, 1986a)
	• Study 2, resistant to natural sunlight in acetone sensitized aqueous solution, ≥84% recovery after 28	(Norris, 1986b)

	days.	
	• Study 3, resistant to simulated sunlight in water at pH 7 at 25°C, ≥92% recovery after 15 days.	(Willems, 1999)
Minimum purity:	99.0%	(WHO, 1991)
Main impurities:	The isomers, alpha- and ß-HCH, are the major impurities	(US-EPA, 2001a)
Appearance:	Colourless, crystalline solid	(WHO, 1991)
Relative density:	1.85 g/cm^3	(WHO, 1991)
Melting range:	>112°C	(WHO, 1991)
Stability:	It has good stability in carbon dioxide, light, air, heat and strong acids; in the presence of alkali or during prolonged exposure to heat; it is dehydrochlorinated leading to the formation of trichlorobenzenes, phosgene, and hydrochloric acid. It is incompatible with strong bases, powdered metals such as Fe, Zn, Al and with oxidizing agents. It can also undergo oxidation when in contact with ozone.	(WHO, 1991)

2.16.2 Formulations

The following formulations (g ai/kg or g ai/l) have been identified from information supplied by the manufacturers: DS (powders for dry seed treatment, 166, 187.5 and 250 g ai/kg); ES (emulsions for seed treatment, 120, 249 and 508 g ai/l); FS (flowable concentrates for seed treatment, 93, 353, 359, 400 and 479 g ai/l); LS (solutions for seed treatment, 69, 106 and 108 g ai/l). These formulations may contain one or more other pesticides (captan, carboxin, diazinon, mancozeb, maneb, metalaxyl, quintozene, thiabendazole, thiram); (US-EPA, 2001a).

2.16.3 Metabolism of Lindane

lindane is metabolized slowly through four possible reactions namely

- Dehydrogenation; which results in gamma-hexachlorocyclohexene
- Dehydrochlorination; which results in gamma-pentachlorocyclohexene
- Dechlorination; resulting in gamma-tetrachlorohexene
- Hydroxylation which also results in hexachlorocyclohexanol

Lindane is metabolised through cytochrome P-450 dependent enzymes. There are over 80 different metabolites of lindane; each with its unique relevance and chemical structure (Macholz & Kujawa, 1985). According to the International Programme on Chemical Safety (IPCS), the essential steps of lindane metabolism are known today (IPCS, 1991). Moreover, lindane increases its own metabolism through the induction of hepatic mixed function oxidase systems (Van Ert & Sullivan, 1992).

Lindane metabolism produces many other substances such as pentachlorophenol which is known to be harmful and chlorinated hydrocarbon epoxide which can cause cancer in animals. A study revealed chlorophenols as the primary urinary metabolites of Lindane excreted by workers involved in Lindane production (Angerer et al. 1983). Other metabolites found in the urine included dihydroxychlorobenzenes, other trichlorophenols, dichlorophenols and tetrachlorophenols (Angerer et al. 1983). Occupational exposure has resulted in identifying pentachlorophenol also as a urinary metabolite in humans (Engst et al., 1978). In vitro investigations also reveal the conversion of γ-HCH by human liver microsomes to five primary metabolite namely 3,6/4,5-hexachlorocyclohexane, pentachlorocyclohexane, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorobenzene (Fitzloff et al., 1982).

This conversion is achieved through dechlorination, dehydrogenation, dehydrochlorination and hydroxylation. Similar in vitro studies have demonstrated that a stable halogenated hydrocarbon epoxide (which may be the cause of the mutagenic and carcinogenic effects of lindane) is formed during the metabolism of pentachlorocyclohexane (Fitzloff et al., 1982).

The metabolism of Gamma-BHC was also studied in 21 workers producing this insecticide. Gas chromatography, electron capture detector and mass spectrometry (GC-ECD and MS) were used in the analysis and the results found 14 Mono-, Di-, Tri- and Tetrachlorophenols in their urine; 7 dihydroxychlorobenzenes of unknown configuration were also detected. 2, 4, 6-, 2, 3, 5- and 2, 4, 5-trichlorophenol were the main metabolites determined (Angerer et al., 1983). Lindane was applied to maize (Zea mays) and upon analysis, the metabolite 1, 2, 3-TCB was observed in maize extracts. Maize plants exposed to gamma-PCCH in aqueous solutions also revealed the metabolites 2,3,5-TCP; 2,4,5-TCP; 1,2,3 4-TeCB; m-DCB; 1,2,4,5-TeCB and 1,2,4-TCB with GC & MS (Menzie, 1980).

2.16.4 Toxicology of Lindane

2.16.4.1 Mode of action

The toxicity of lindane is exerted mainly by stimulating the central nervous system (Joy, 1982). Rhabdomyolysis which is a direct effect of lindane on striated muscle has been demonstrated in humans (Munk & Nantel, 1977). The vascular endothelial lining may also be affected by high doses of lindane; causing disseminated intravascular coagulation (West, 1967; Sunder et al., 1988). Lindane interferes with the gamma-aminobutyric acid (GABA) system in the nervous system. Seizures caused by lindane can be said to be provoked by GABA-A mimetic. Other mechanisms by which lindane may cause neurological effects in animals include changes in GABA functional activity, enhanced synaptic activity, and inhibition of sodium ions, potassium ions and ATPase (ATSDR, 2005, Ratra et. al, 2001).

Lindane acts in the liver by interfering with glutathione metabolism and hepatic oxidative capacity. It also Inhibits Mg^{2+}ATPase activity in rat liver tissue; this indicates ATPase enzyme sensitivity to the action of lindane. Researchers suggested after the above observations that some toxic effects exhibited by mammals exposed to Lindane is the result of lindane interacting with ATPase activity (ATSDR, 2005).

2.16.4.2 Toxicity

The Fatal dose of lindane in an adult human is approximately 10 to 30 g. However; doses of 1.6 g and 45 g can produce seizures in children and adults respectively. Serum lindane concentrations of 1.3 mcg/mL have caused deaths (Kurt et al, 1986) while concentrations up to 0.60 mcg/mL have resulted in hypertension, muscle weakness, acidosis, myoglobinuria, seizures and acute renal failure. Headache, dizziness, nausea, lethargy and sometimes seizures have been observed in children after ingesting 150 - 450 mg of lindane (Kurt et al, 1986). After 1 or 2 days dermal exposure of lindane, muscle tone and posturing may appear. Seizures can also occur with such exposure. Sub-chronic dermal and oral exposures combined have resulted in vomiting and seizures (Lee *et al*., 1976; Wheeler, 1977).

2.16.4.3 Acceptable daily intake

In an 18- week study in rat diet, 0.33 mg/kg/day was reported as the no observed adverse effect level (NOAEL) (IRIS, 1997). The FAO/WHO, (1978), established an ADI of 0.008 mg/kg body weight. Moreover, based on degenerative renal tubules and hypertrophy of the liver, 1.55 mg/kg/day was also reported as the lowest observed adverse effect level (LOAEL) (IRIS, 1997). The U.S. EPA has also established an RfD of 3×10^{-4} mg/kg/day for lindane (U.S. EPA, 1998). This value was based on a 0.33 mg/kg/day NOAEL. Rats used were exposed for 12 weeks while an uncertainty factor of 1000 to account for interspecies variation as well as protect sensitive human subpopulations was used (Teuschler et al., 1999).

2.16.5 Clinical Effects of Lindane

In instances of acute poisoning, route of absorption of lindane does not have much influence on its clinical effects; it only delays the manifestation of signs and symptoms. Ingestion of lindane results in nausea, vomiting, hypersalivation, and occasionnally diarhoea; convulsions could occur without previous signs or symptoms (Jaeger et al., 1984; Munk & Nantel, 1977). Lindane is well absorbed through the inhalation route, though there are yet no reported cases of its acute poisoning in the literature reviewed through this route (Milby et al., 1968). Dermal exposure may occur after application without personal protective equipment. In this type of exposure, convulsions could be the first sign of toxicity (Tenenbein, 1990; Rasmussen, 1981). Exposure of lindane to the eye also causes eye irritation, though literature reviewed showed no data for parenteral exposure. In mild to moderate exposure, one or a few episodes of convulsions may occur without any other clinical effects and sequelae. Death through Lindane exposure may occur as a result of disseminated intravascular coagulation, brain edema and cardiac arrest (Sunder et al, 1988). Renal failure and rhabdomyolysis are generally non-fatal (Munk & Nantel, 1977; Jaeger et al, 1984).

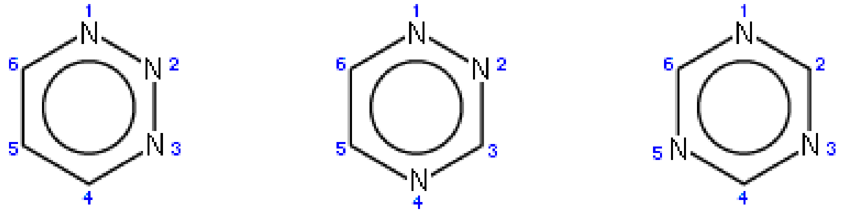
2.16.5.1 Systemic description of clinical effects

Prolonged convulsions may be followed by cardiac arrest in the cardiovascular system (Davies et al., 1983) while respiratory depression may be secondary to these convulsions (Davies et al., 1983). Broncho-aspiration pneumonitis may follow the ingestion of mixtures of Lindane and hydrocarbon- based solvents (Jaeger et al, 1984).

In the central nervous system (CNS), convulsions, confusion, unconsciousness, and coma are the main toxic effects of Lindane (Davies et al., 1983; Jaeger et al., 1984; Kurt et al., 1986; Munk & Nantel, 1977). Descriptions of chronic psychological and neurological complications have also been made after severe lindane poisoning (Hall & Hall, 1999). Paresthesias, hyperreflexia, numbness of extremities have also been reported as the main toxic effects of lindane poisoning in the peripheral nervous system (Jaeger et al, 1984, Davies et al., 1983). In the skeletal and smooth muscles, Rhabdomyolysis (i.e., a direct effect of lindane on striated muscle) has been described in a few cases through Electromicroscopy studies (Jaeger et al, 1984; Munk & Nantel, 1977). Lindane is thought to impact pregnancies in the reproductive and endocrine systems; this may result to miscarriages in certain cases (Konje et al., 1992; Gerhard et al., 1998,). Elevated liver enzymes have been described after acute exposures in the hepatic system in a work by the IPCS; renal insufficiency may also occur as a consequence of rhabdomyolysis (IPCS, 1990; Munk & Nantel, 1977, Jaeger et al., 1984,).

2.17 Triazine

Triazines refer to a class of nitrogen-containing ring structure with a parent molecule whose molecular formula is $C_3H_3N_3$. They have a planar six-membered benzene-like three of the carbons replaced by nitrogens. It has three isomers that can be distinguished by the positions of their nitrogen atoms and are referred to as 1, 2, 3-triazine, 1, 2, 4-triazine, and 1, 3, 5-triazine. Most of them are used in selective weed control programmes. Those with nonselective properties such as prometo are used on industrial sites. Triazines inhibit electron transfer in photosynthesis though not all members of this family are herbicides. Atrazine, hexazinone, metribuzin, prometon, prometryn, and simazine are herbicide members of this family. Research on the herbicidal capacity of triazines started in the early 1950s putting them among the world's oldest herbicides. Atrazine is widely used in maize production and selective weed control in sugarcane and sorghum. Hexazinone is used primarily in industrial sites such as in alfalfa, pastures and pine plantations. Metribuzin, due to its different chemical properties, has diverse use patterns from the other triazine herbicides. Some of these uses include selective control of broadleaves in soyabean and some vegetables. Prometryn is normally used on cotton and celery. Simazine is normally used in citrus weed control while its specific uses such as on grapes and certain berries, are classified as restricted. Product formulations of the triazine pesticides vary widely. Triazines exist in three isomeric forms with 1, 3, 5-triazines, being the most common. These isomers are shown below:



1,2,3-triazine

1,2,4-triazine

1,3,5-triazine
or
s-triazine

Figure 2.2: Isomers of triazines

2.17.1 Atrazine

Atrazine is a triazine herbicide. It is a selective systemic herbicide which acts by inhibiting photosynthesis and interfering with other enzymic processes (MAFF, 1993). It was manufactured in 1958 by J.R. Geigy, who is now part of Novartis; atrazine’s largest manufacturer (Hicks, 1998). It is now being manufactured by a host of other companies with a range of trade names that include Atrazol (Sipcam), Coyote (Defensa), Atrazina (Cequisa), Marksman (Novartis) and Vectal (Aventis)(Tomlin, 2000). It is used to control pre and post-emergence annual and broad leaved weeds and perennial grasses (Tomlin, 2000). When applied, it is absorbed through the plant roots though it can also enter through the foliage and accumulate in the apical meristems and leaves (MAFF, 1993). Atrazine is produced commercially in the United States as a technical-grade chemical with a purity of 92–97% (IARC, 1999) to 99.9% (USEPA, 1983) active ingredient. Impurities in the formulation included dichlorotriazines, hydroxytriazines, and tris (alkyl) aminotriazines.

Atrazine is used worldwide mainly in maize production (Hicks, 1998). It is also used in the production of sorghum, sugar cane, pineapples and for industrial weed control (Tomlin, 2000). Atrazine use in Europe is concentrated on maize, orchards and vineyards (MAFF, 1993). In the United Kingdom, its use is mainly for maize production (Tomlin, 2000). In 2004, it was banned in the European Union because of persistent groundwater contamination (ECHA, 2014). It has gained much attention because of its frequent detection in surface and groundwater supplies and though most countries have banned the usage of atrazine due to health risk and associated environmental contamination, it is still used in Ghana.

2.17.1.1 Identity of Atrazine

Atrazine is a white crystalline solid, odourless with a melting point of 172-175 ^{o}C, density of 0.35 g/mL, octanol/water partition coefficient (log Kow) of 2.7645, and vapor pressure of 40 KPa at 20^{o} C. Atrazine is moderately soluble in water (33 ppm at 25 ^{o}C), and is soluble in octanol (0.82 g/100 mL), ether (0.86 g/100 mL), methanol (1.4 g/100 mL), ethyl acetate (2.5 g/100 mL), and chloroform (7.8 g/100 mL) at 20 ^{o}C (U.S EPA, 2006). Atrazine is prepared by reacting cyanuric chloride with one equivalent of ethylamine; followed by one equivalent of isopropylamine in the presence of an acid binding agent (Sittig, 1980). It has a solubilities of 183 g/kg DMSO; 52 g/kg chloroform; 28 g/kg ethyl acetate; 18 g/kg methanol; 12 g/kg diethyl ether; 0.36 g/kg pentane (Lide and Milne, 1994). It also has a solubility of 34.7 mg/L at 26 deg C in water (Ward and Weber, 1968). It has Hazardous decomposition products which may include but are not limited to carbon monoxide, hydrogen cyanide, and acetonitrile (Sipcam Agro, 2011). Atrazine also emits toxic fumes of hydrogen chloride and nitrous oxides when it is heated to decomposition temperature (Lewis, 2004). It is formulated as liquid, water dispersible granule, dry flowable or wettable powder (Mester and Sine, 2011).

ISO common name: Atrazine

Chemical name:

IUPAC: 6-chloro-*N*^{2}-ethyl-*N*^{4}-isopropyl-1, 3, 5-triazine-2, 4-diamine or 6-chloro-4-N-ethyl-2-N-propan-2-yl-1, 3, 5-triazine-2, 4-diamine (PubChem, 2014).

Identification numbers	**References**
CAS registry : 1912-24-9	(HSDB 2002)
NIOSH RTECS : XY5600000	(HSDB 2002)
DOT/UN/NA/IMO UN : 2763	(HSDB 2002)
UN :2997	(HSDB 2002)
UN :2764	(HSDB 2002)
UN :2998	(HSDB 2002)
IMO :6.1	(HSDB 2002)
IMO :3.2	(HSDB 2002)
HSDB :413	(HSDB 2002)
Experimental code number: G-30027 (Ciba-Geigy)	Farm Chem (2001)

Synonyms and trade names:

Aatrex, Aatram, Atratol, Gesaprim, 6-Chloro-n-ethyl-n'-(1-methylethyl)-1, 3, 5-triazine-2,4-diamine, 2-Chloro-4-ethylamino-6-isopropylamine-s-triazine, Aktikon, Crisatina, Fenamin, Hungazin, Weedex A, Zeazin (CDC-ATSDR,2001)

Structural formula: (USEPA, 2003)

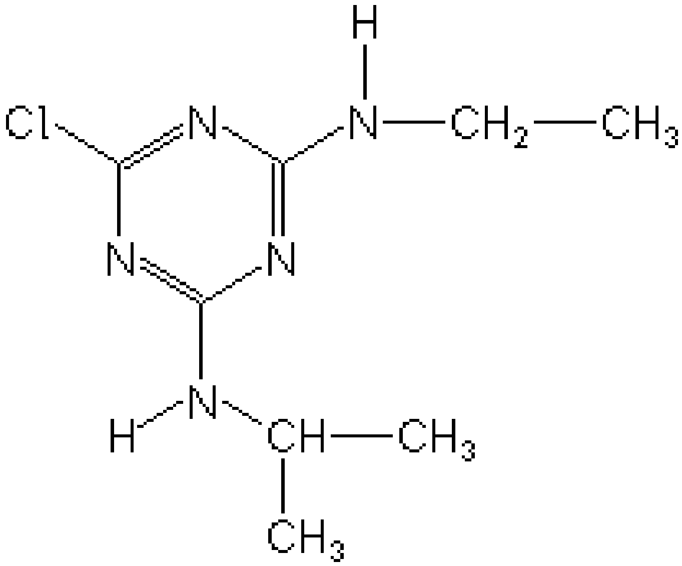


Figure 2.3: Structure of Atrazine

Molecular formula: $C_8H_{14}ClN_5$
Molecular weight: 215.68 g·mol^{-1} (USEPA, 2003)

PHYSICOCHEMICAL PROPERTIES

Table 2.7 gives a summary of some physical and chemical properties relevant to atrazine's environmental fate:

Table 2.7: Physical and chemical properties of atrazine

Property	**Value**
Melting point	175–177 °C
Density	1.187 g/cm3 at 20 °C
Water solubility	30 mg/l at 20 °C
Log octanol- water partition coefficient	2.3
Vapour pressure	40 × 10−6 Pa at 20 °C

Source: Meister (1989); Royal Society of Chemistry (1991); Worthing (1991).

2.17.2 Environmental Fate of Atrazine

Atrazine persists in surface and groundwater with a half-life of longer than 6 months, with some tendency to bind to sediment (US-CDC-ATSDR, 2003). Atrazine has a slight tendency to bioaccumulate in some aquatic organisms, including invertebrates and fish (US-CDC-ATSDR, 2003). Atrazine persistence in soil generally ranges from 14-109 days, though in some soils can persist to at least 4 years (USEPA, 2003; US-CDC-ATSDR, 2003). Atrazine can be found in the particulate and vapor phases of air following application, and can be transported up to 186 miles from the site of application (US-CDC-ATSDR, 2003). In air, atrazine can be degraded by reacting with hydroxyl radicals (US-CDC-ATSDR, 2003).

2.17.3 Absorption, Distribution, Metabolism, and Excretion of Atrazine

The absorption, distribution, metabolism, and excretion of atrazine has been investigated in humans and animals.

2.17.3.1 Absorption

The only available inhalation toxicity studies in humans involved occupational exposure to very large atrazine particles (30–70 µm) (Catenacci et al., 1990, 1993), which made it unlikely that any significant amount of atrazine reached the lungs. Evidence of absorption following oral exposure was provided by a single case report of a man who ingested a weed killer containing atrazine and other chemicals; atrazine was detected in the plasma and several organs at autopsy (Pommery et al., 1993). Absorption of atrazine following dermal exposure has been evidenced by the presence of atrazine and its metabolites in urine of people exposed to radiolabelled Aatrex (a commercial product containing atrazine) via a forearm patch (Buchholz et al.1999), and in urine of workers exposed primarily via dermal contact (Catenacci et al., 1990, 1993). An in vitro study using human skin samples also indicated that limited absorption (16.4% in 24 hours) occurs through the skin (Ademola et al., 1993). Further evidence of absorption following oral (Meli et al., 1992; Timchalk et al., 1990) and dermal (Hall et al., 1988) exposure to atrazine has been provided by animal studies showing the presence of atrazine and its metabolites in the plasma, urine, and/or faeces. Absorption following gavage administration has been described as a first-order process with an absorption half-life of 2.6 hours (Timchalk et al., 1990), with 37–57% of the administered dose recovered in the urine and 14% in the faeces (Meli et al., 1992; Timchalk et al., 1990).

2.17.3.2 Distribution

Data on distribution of atrazine in humans after oral exposure was limited to a single case report of a 38-year-old man who died of progressive organ failure and shock 3 days after ingesting 500 mL of a weed killer that contained 100 g atrazine, 25 g of aminotriazole, 25g of ethylene glycol, and 0.15 g of formaldehyde (Pommery et al., 1993). At autopsy, atrazine was detected in the kidney, small intestine, lung, liver, pancreas, muscle, heart, and plasma. Radioactivity was detected in the plasma, whole skin, and carcass of rats gavaged with 30 mg/kg [C14]-atrazine (Timchalk et al., 1990), and in the liver, brain, heart, lung, kidney, digestive tract and skeletal muscle of rats gavaged with up to 17.7 mg/kg [C14]-atrazine (Bakke et al., 1972).

2.17.3.3 Metabolism

Atrazine is primarily metabolized in humans via dealkylation, probably followed by glutathione conjugation and conversion to mercapturic acids. This is apparently true regardless of route of exposure (Buchholz et al., 1999; Catenacci et al., 1993; Meli et al., 1992; Timchalk et al., 1990). In vitro studies using microsomal preparations from liver and other tissues of humans and animals have indicated that dealkylation of atrazine is mediated by cytochrome P-450 enzymes (CYPs) (Adams et al., 1990; Ademola et al., 1993; Croce et al. 1996; Hanioka et al., 1998a, 1999; Lang et al. 1996, 1997; Meli et al., 1992; Venkatesh et al., 1992). In humans, the primary CYP responsible for phase I metabolism is probably CYP1A2 (Lang et al., 1997), and in rats, CYPs 2B1 and 2C11 have been implicated as the primary metabolic enzymes (Hanioka et al., 1998a). Available data indicate that phase II metabolism of Atrazine proceeds through glutathione conjugation and mercapturic acid formation (Adams et al., 1990; Egaas et al., 1995).

A pesticide’s metabolism may play a very significant role in its toxicity. Several works have investigated the human hepatic metabolism of Atrazine. However, further exploration is necessary since its metabolic pathways in humans are not fully understood (Joo et al., 2010). Atrazine has four hydroxyatrazine compounds and three chlorinated atrazine compounds as metabolites. The three chlorinated metabolites are desethylated Atrazine (DEA), desisopropyl Atrazine (DIA), and diaminochlorotriazine (DACT) (U.S EPA, 2003). In studies by Lang and hodgeson using human liver microsomes (HLM), desethylatrazine (DEA) and desisopropylatrazine (DIA) were found as major metabolites while 1-hydroxyisopropylatrazine (HIATZ) was a minor metabolite (Lang et al., 1996;

Lang et al., 1997; Hodgson et al., 2008). Lang et al., 1997, have also reported that the major CYP isoform in the oxidative phase 1 metabolism of s-triazine, including Atrazine (ATZ) in human liver is cytochrome P450 (CYP) 1A2. Other studies of human exposure to ATZ revealed other ATZ metabolites such as didealkylated Atrazine (DIDEA), hydroxyatrazine (HA) and mercapturic acid conjugates (Panuwet et al., 2008; Lucas et al., 1993; Jaeger et al., 1999; Olsson et al., 2004).

Below is a diagramatic representation of a proposed in vitro metabolism of Atrazine in pooled human human liver fractions: Human liver microsomes (HLM); Glutathione (GSH); Human liver S9 (HL S9) (Joo et al., 2010).

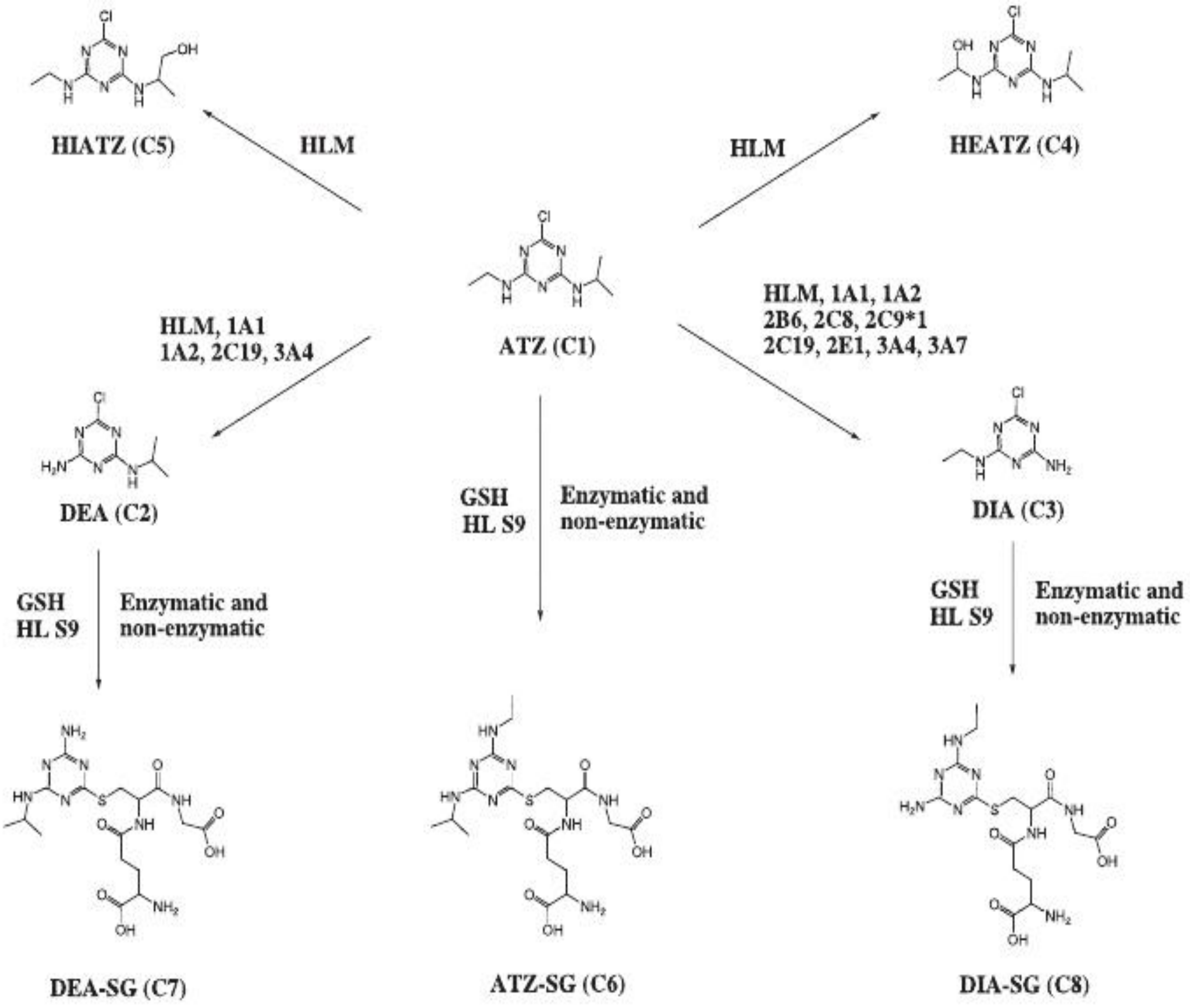


Figure 2.4: Metabolism of atrazine

DEA and DIA, are the initial metabolites of atrazine which are then rapidly further metabolized to DACT. Diaminochlorotriazine (DACT) is the predominant metabolite resulting from mammalian exposure to atrazine. In a study in which gas chromatography was used to identify deethylated, deiso-propylated and di-dealkylated atrazine, as well as atrazine itself, in the urine of atrazine manufacture workers, di-dealkylated atrazine represented 80% of the urinary metabolites (IARC,1972)

2.17.3.4 Excretion

In humans, 50% of all urinary atrazine metabolites were excreted within 8 hours and 100% within 24 hours (Catenacci et al., 1993). In rats, approximately 57% of the radioactivity from administered (14C)-atrazine was excreted in the urine within 24 hours (Timchalk et al., 1990), and urinary atrazine metabolites decreased to 1/30 or less of the 24-hour level by 48 hours post exposure (Meli et al., 1992). Specific data on elimination and excretion of atrazine by any route were limited. However, the primary route of excretion appears to be in urine, as indicated by the detection of urinary atrazine and its metabolites in a number of species exposed via oral and dermal routes (Bakke et al., 1972; Buchholz et al., 1999; Catenacci et al., 1990, 1993; Meli et al., 1992; Timchalk et al., 1990). Faecal excretion was a minor route (Buchholz et al., 1999; Timchalk et al., 1990).

2.17.4 Toxicology and Health Effects of Atrazine

2.17.4.1 Mode of action

Atrazine exerts its toxicity mainly in the endocrine system. Several studies have reported atrazine as being an endocrine disruptor which can cause hormonal imbalance (USEPA, 2007). In 2006, the USEPA classified atrazine as not likely to cause cancer. Though there is sufficient evidence in experimental animals for the carcinogenicity of atrazine, there is inadequate evidence in humans. Some effects reported in adults (human and experimental animals) include ovarian histopathology (changes in ovarian tissue), attenuation of the LH (leutenizing hormone) surge, shortening of estrous cycle length, decreases in pituitary hormone levels and liver effects such as liver histopathology, increased liver enzymes and serum lipids (US-CDC-ATSDR, 2003). Moreover, effects on the immune system, central nervous system and cardiovascular function have been reported in adults (US-CDC-ATSDR, 2003). Atrazine exposures may also be linked to some types of non-Hodgkin's lymphoma in adult humans (USEPA, 2003). Intrauterine growth retardation, decreased birth weight and increased risk of preterm delivery were significantly associated with atrazine concentrations in drinking water (Munger et al., 1997; Villanueva et al., 2005). Several experimental animal studies reported reproductive and endocrine effects following atrazine exposure (Stoker et al., 2002; Stoker et al., 1999; Rayner et al., 2005). Reproductive studies with experimental animals (Stoker et al., 2000; Kniewald et al., 2000) reported increased incidence of preterm delivery and intrauterine growth retardation. Delayed onset of puberty occurred in young male (Stoker et al., 2000; Stoker et al., 2002) and female (Laws et al., 2000; Rayner et al., 2004; Chapin et al., 1996) rats exposed to atrazine. Atrazine exposures could also be associated with mammary tumors in at least one strain of adult rats (Chapin et al., 1996). Atrazine's Chlorinated metabolites such as diethylatrazine (DEA), di-isopropyl-atrazine (DIA) and diaminochlorotriazine (DACT)) are generated in water, soil, and animal tissues; these metabolites are considered equal in toxicity to atrazine (USEPA, 2003).

2.17.4.2 Exposure routes

Occupational exposure to Atrazine may occur through direct dermal contact with this compound or inhalation at Atrazine production workplaces. In a NIOSH study dubbed NOES Survey 1981-1983; it was estimated that 1,001 workers were potentially exposed to atrazine in the US (NIOSH, NOES, 2011). The NOES (National Occupational Exposure Survey) work, however, does not include farm workers and applicators. This implies that the actual occupational exposure to atrazine will be much higher than predicted in this survey. Oral exposure may also occur through ingestion of contaminated food and water

2.17.4.3 Average daily intake

The average daily intake of atrazine in the diet of Belgians from a study conducted between 1991 and 1993 was determined to be 0.0007 mg/kg/day (Dejonckheere et al., 1996). The mean daily intake of atrazine in the United States from a study conducted between 1984 and 1986 was determined to range from 0.0033 to 0.0123 ug/kg body wt/day depending upon age (Gunderson, 1995).

2.18 Dietary Intake

Pesticides are an integral part of modern food production and consumers are aware that they ingest pesticides with their food. One of the key issues that interests both regulators and consumers is the question of how much pesticide is consumed. The amount of pesticide consumed is termed dietary intake, or simply intake.

2.19 Analytical Techniques

A number of analytical techniques are employed for pesticide residue analysis. The major ones include conventional methods like High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) (Pinto and Jardim, 2000) and the radiometric techniques using radionuclide as tracer (Hussein *et al.*, 2001). Analytical procedures employed for pesticide residues include: sample preparation, the extraction of the pesticide, clean-up of the sample extract and the analysis of the pesticide residues using the appropriate instrument e.g. Gas Chromatography.

The Gas Chromatography (GC) is one of the most suitable facilities in pesticide residue analysis due to its high versatility, sensitivity and selectivity. Some of the detectors include: Nitrogen Phosphorous Detector (NPD); Atomic Emmission Detector (AED); Mass Spectrometry (MS); Electron Capture Detector (ECD) and Flame Photometric Detector (FPD). The Electron Capture Detector is the most used in pesticide residue analysis. It demonstrates a very sensitivity to molecules containing highly negative atoms such as the halides. It is therefore the best for the determination of trace levels of organochlorine pesticides (Baird, 1997). Although, it has a very good sensitivity to polychlorinated hydrocarbons and other halogenated pesticides but its selectivity is very poor, this is simply because all kinds of electron attracting functional groups such as aromatic structures and nitro groups also give a response on this detector (Torres *et al*., 1996).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter gives an account of the various research methodologies that were employed. It entails the following: the study area, sample collection, extraction, clean- up and analysis of sample using GC to ascertain the levels of lindane, atrazine and other organochlorine pesticide residues in maize in Ghana, equipment and chemicals used, and questionnaire administration.

3.2 Study Area

The study area occupied major maize production zones in Ghana. Nine out of the ten regions in Ghana with production figures of 60,000 metric tons and above were sampled. These are Brong Ahafo region with 466,208 MT; Eastern region with 399,959 MT; Ashanti region with 201,786 MT; Northern region with 170,512 MT; Central region with 168,120 MT; Upper west region with 122,254 MT; Volta region with 84,262 MT; Western region with 82,430 MT and Upper east region with 64,434 MT per annum (MOFA, 2014).

3.2.1 Brong Ahafo Region

This is the largest maize producing region of Ghana with annual production figures of 466,208 MT per annum in 2013. It lies between latitudes 8^{o}45’N to 7^{o}30’ S and longitudes 0^{o}15’ E to 3^{o} W in the west central part of Ghana. It is bounded to the north by the northern region and south by the Ashanti and Western regions. It is also bounded to the east by the Volta region and south-east by the Eastern region. It shares boundaries to the west with Cote D’Ivoir. The central point of the land mass of Ghana is located in this region (Kintampo). The moist semi-deciduous forest vegetation dominates the southern parts of the region while the transitional zone is the main vegetation in the middle parts of the region. The guinea savanna woodland vegetation also dominates the northern parts of the region. The three main soil groups found in the region are forest ochrosols covering the south-western parts, savanna ochrosols stretching widely from the west and narrows towards the east and ground water laterite ochrosols intergrade also found in the northern parts of the region. These vegetation zones and soil types favour the cultivation of a variety of food crops including maize, rice and cassava as well as export crops such as cocoa. The Black Volta, Tano, Bia, Pra and Tain rivers form the main drainage systems in the region. Rainfall in the region decreases from the south to the north. The average annual rainfall in the southern parts is 1651 mm which decreases to between 1143-1270 mm in the north; while the average annual total rainfall is between 1088-1197 mm. the geology of the region is mainly of the Voltaian Supergroup to the eastern parts composed of quartzite, shale, arkose and mudstones and the Birimian Formation to the western parts. The eastern and southern parts of the region are at elevations of about 152 m above sea level gradually increasing to about 534 m in the northern parts. However, the regional capital, Sunyani is about 384.8 m above sea level while other high altitude areas in the region are Bosumkese 712.6 m, Bonsam 643.1 m, Asuakwaw 483.7m, Sampa 547.5 m and Banda 592.2 m. The average temperature in the region is about 23.9 ^{o}C with relative humidity of over 75 % throughout the year.

3.2.2 Eastern Region

This region has annual production figure of 399,959 MT in 2013. It shares borders to the north with the Brong Ahafo Region, to the north – west, it shares borders with the Ashanti Region, to the east with the Volta Region and to the south-east and west with the Greater Accra and Central Regions respectively. The eastern region lies between longitude 0^{o} 30' East and 1^{o} 30' West and latitude 5^{o} 30' N and 7^{o} 22' N. It has fairly moderate humidity between 65 and 95% with annual average temperature between 22 and 33 ^{o}C. It has an undulating topography which consists of vast flat lands in the Afram Plains and highlands including the Kwahu scarps, Atiwa- Atweredu Range and Akuapem Range. Three main vegetation zones are found in the eastern region namely: Semi-deciduous rain forest, Transitional savanna zone and Coastal savanna. Lithosols and forest ochrosols are the main soil types in the region. However, savanna ochrosols dominate Afram plains, Yilo-krobo, Manya and Asuogyaman districts. Savanna ochrosol occurs mainly in Kwahu North and parts of Yilo-Krobo, Manya and Asuogyaman districts. Patches of forest rubisol is also found in the East Akim and New Juaben districts. Major rivers found in the region include Afram, Densu, Pra, Birim and the volta lake. Two main rainy seasons occur in this region. The major one is from March to July while the minor one begins in September and ends in November. The annual rainfall in the forest zone ranges between 1500 mm to 2000 mm while this figure decreases to between 900 mm to 1300 mm in the savanna zone. The Dry season also begins in November and ends in February and is normally characterized by cold dry weather.

3.2.3 Ashanti Region

It is the third largest maize producing region in Ghana with 201,786 MT in 2013. The region lies between longitude 0^{o} 15' – 2^{o} 25' West and Latitude 5^{o} 50' – 7^{o} 40' North. It shares borders to the north with Brong Ahafo region, west with the Western region, south with the central region and east with the Eastern and Volta regions. The region has a total land area of 24,389 square kilometer; about 1,463,340 hectares (60 %) of which are arable and 1,181,788 hectares (81%) of these arable lands are cultivated. The drainage systems found in this region are the rivers Pra, Offin, Afram, Oda and a 47.68 sq km meteoritic lake called Lake Bosomtwe. Two main vegetation zones dominate the region. The moist semi-deciduous forest dominates the southern parts and the northern parts are mainly covered by the guinea savanna. However, streams in the savanna zone and the Afram River have dominant riverine forest vegetation. Savanna ochrosols and forest ochrosols are the two main soil types found in the region. The average annual rainfall in the region is between 1,100 mm and 1,800 mm with average annual temperature ranging between 25.5 ^{o}C with 85 % humidity and 32 ^{o}C with 65 % humidity in the southern and northern parts respectively. The rainfall, temperature, and the rivers can support all year agricultural production of food crops such as maize, cassava, plantain, rice, yam, cocoyam and cash crops such as cashew, cocoa, citrus coffee, oil palm, and mango. Maize production is dominat in Ejura Sekyeredumase.

3.2.4 Northern Region

In terms of land area, this is the largest region in Ghana. It shares borders to the north with the Upper East and the Upper West regions; to south with the Brong Ahafo and Volta regions; to the east with Republic of Togo and to the west with Cote d'Ivoire. Its coordinates are latitude 9°30'0" N and longitude 1°0'0" W. It is Ghana's 4th largest maize producer with just over 170,000 MT in 2013 (MOFA, 2014). The region has a generally low-lying topography with only the Gambaga escarpment as the high land area. The White and Black Volta and their tributaries constitute the main drainage system in the region. The region has a relatively dry climate with annual rainfall figure varying from 750 to 1050 mm. the rainy season starts in May and ends in October while the dry season characterized by the harmattan winds starts in November and ends in March or April. The region has maximum annual mean temperatures of about 40 °C and minimum temperatures of about 14 °C. The main vegetation is characterized by large stretches of grassland areas interspersed with the guinea savannah woodland.

3.2.5 Central Region

This region is bordered to the north by Ashanti region, to the north-east by the Eastern region, south-east by the Greater Accra region and to the west by the Western region while the Gulf of Guinea covers the southern part of the region. It is the fifth largest maize producer with 168,120 MT in 2013. Though one of the smallest regions in Ghana, it has the longest coastline of 150 km. The region has a major rainy season with stretching from April to July with average rainfall figures of 1500 mm. the minor rainy season spans from September to November with rainfall average of 800 mm. It has annual temperature ranging between 24 ^{o}C and 34 ^{o}C and relative humidity of between 50 % and 85 %. The region has a total cultivable land area of about 7,864 km^2 (about 80 % of the Region's Total Land Area) with 40 % under cultivation.

3.2.6 Upper West Region

The Upper West region lies between longitudes 1^{o}25’W and 2^{o}50’W, and between latitudes 9^{o}35’N and 11^{o}N. It shares borders with the Northern region to the south, Upper East and Northern regions to the east and Burkina Faso to the north and west. It is the sixth largest maize producer with 122,254 MT in 2013 (MOFA, 2014) with five districts producing over 10,000 MT per annum. The Geology of the region is characterised by Birimian and post-Birimian granites with Fluvisols, Arenosols, Lixisols, Vertisols or Gleysols forming the main soil types in the region (FAO classification system). Two major rivers are found in the region. These are the Black Volta which flows along the western end of the region and the Kulhpong River flowing along the Eastern end. Altitude in the region ranges from the low lying Black Volta which is 200 m to about 350 m for the ridge that stretches from Wa in the south to the Burkina Faso border in the north. The region has a short rainy season with annual mean rainfall of about 900 mm in Tumu and about 1111 mm in Wa to the south with relative humidity of 69 %. The dry season is characterised by the harmattan winds with relative humidity of 16 % in Wa. This rainfall pattern is a determinant in the two main agro-ecological zones in the region. The Sudan savanna zone covers the northern and north eastern parts. This vegetation zone has scattered trees with sparse ground cover of grasses. Trees such as Baobab (*Adansonia digitata*), Dawadawa (*Parkia clappertoniana*), shea (*Butyrospermum paradoxum*) are abundant. The Guinea savanna zone also covers the southern parts of the region with trees such as shea, Dawadawa and Mahogany dominant. Andropogon, Cymbopogon spp. and Forbes are grass species that dominate the south during the wet season (Soil Conservation and Water Management Division, UWR, unpublished).The annual mean temperature for Wa is about 27.2 °C with a mean maximum of about 35.5 °C and mean minimum of about 18.8 °C.

3.2.7 Volta Region

The Volta region is located at latitude 3^{o} 45' N and longitude 8^{o} 45' N. Geographically, it lies at the eastern side of Ghana and bordered by four regions namely, Greater Accra, Eastern, Brong Ahafo and Northern regions. The vegetation of the region can be categorized as coastal strand Mangrove Swamps, woodland Savannah, Savannah grassland, Mangrove Swamps and Deciduous forest. It had annual maize production of over 84,000 MT in 2013 (MOFA, 2013). It has a low-lying altitude of less than 15 meters above sea level at the coast to 855 meters with mountain Afadza being the highest point. The Volta Lake drains a substantial portion of the region together with other rivers (Daka, Oti, Dayi, seasonal streams like Kplikpa, Aka and Agali). Sandy coastal soils, savannah ochrosols, regolistic groundwater laterites, topohydric and luthochronic earth are soil types dominating the region. It has a tropical climate characterised by moderate temperatures of 12 ^{o}C to 32 ^{o} C for most of the year with bi-modal rainfall pattern. The minor rainy season starts from March and ends in July with annual rainfall of 513.9 mm while the major rainy season begins in mid-August and ends in October with annual rainfall of 1099.88 mm.

3.2.8 Western Region

This region is the eighth largest producer of maize in Ghana with over 80,000 MT in 2013. Its coordinates are latitude 5°30'0" N and longitude 2°30'0" W. It shares boundary to the east with the Central region, to the west with the Ivory Coast, to the north with

Ashanti and Brong-Ahafo Regions, and to the south with the Gulf of Guinea. The region lies in the equatorial climate zone which is characterised by moderate temperatures. It is the wettest region in Ghana with average annual rainfall figures of 1600 mm. it has about 75 % of its vegetation within the forest zone.

3.2.9 Upper East Region

The upper East region shares borders to the north with Burkina Faso, to the south with the Northern Region and to the East and west with Togo and the Upper West Region respectively. Its location by coordinates is at Latitudes 10^{o}15' and 10^{o}10'N and Longitudes 0^{o} and 1^{o}4'W. It is the nation's ninth largest maize producer with just over 64,000 MT in 2013. Gentle slopes dominate the topography of the region. Erosion is predominant in the region with about 70 % of the region subject sheet and gully erosion. The valley areas of the region have soils ranging from sandy loams to salty clays. These soils are easily water logged and hence cause seasonal flooding. The White and Red Volta and Sissili Rivers are the main drainage systems in the region (Regional Coordinating Unit, 2003). The average rainfall of the region is 921 mm with ranges between 645 mm and 1250 mm. the distribution of rainfall in the region is unimodal with a single 5 to 6 months growing season and 6 to 7 months long dry season. The harmattan winds dominate the dry season with low humidity and temperatures. Average annual minimum temperatures of 15 ^{o}C have been recorded and maximum of 45 ^{o}C. The woodland savannah is the dominant vegetation in the region. It is dominated by short and scattered drought-resistant trees and grass which lies within the Guinea-Savannah zone. However, some areas around Bawku have the Sudan-Savannah vegetation. Groundnut, millet, guinea-corn, maize, beans and sorghum are the main produce of the region.

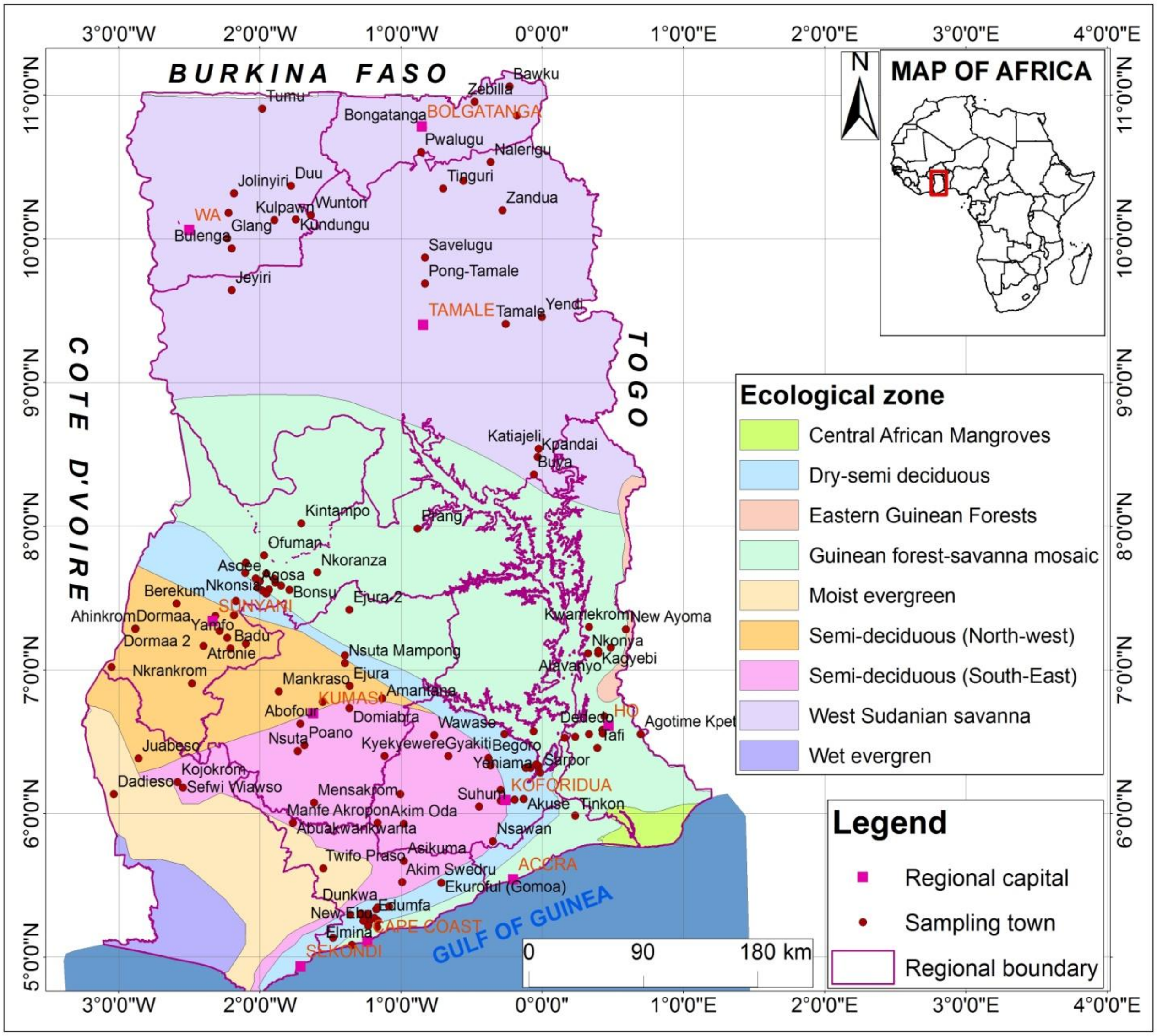


Figure 3.1: Map of sampling area

3.3 Field Survey

Assessment of the knowledge, practice and attitudes regarding safe use of atrazine, lindane and other organochlorines, toxicity awareness and symptoms among farmers and traders was done by administering questionnaires. Face-to-face interviews with farmers, farm workers, traders and field observations during farming activities were also employed in data acquisition. The questionnaire consisted of structured, semi-structured and unstructured items with data including social and demographic characteristics of farmers, farm practices, pesticide use practices, availability and safe use, toxicity awareness and symptoms among farmers being collected. The questionnaire was designed in English but was translated into the various local languages of the study areas. Leading questions were avoided to check bias.

Figure 3.2: Face-face interview with a farmer at Odumase, Sunyani in the Brong Ahafo region of Ghana, also shows inspection of pesticide mixture in tank of knapsack sprayer

3.4 Sampling

Dry Maize samples (1 kg each) were collected from Brong Ahafo region (30 samples), Eastern region (20 samples), Ashanti region (15 samples), Central region (14 samples), Northern region (11 samples), Volta region (15 samples), Upper West region (8 samples), Upper East region (5 samples) and Western region (2 samples). These regions were selected based on maize production figures of at least 60,000 metric tonnes per annum (60,000 – over 400,000 MT per annum). This selection criterion was based on data collected from the Statistic Research and Information Directorate of the Ministry of food and agriculture (MOFA, 2014). Maize samples were bought at major markets in the selected regions. The samples collected were then put in to hermetic polyethylene bags and labelled appropriately. At each sampling site, five samples (1 kg each) were collected and mixed thoroughly before a representative 1 kg sample is bagged for that town and labelled. Sampling began in October 2014 and ended in April 2015. Maize sampling was done simultaneously with interviewing and questionnaire administration.

Figure 3.3: Drying Maize samples at Sarpor in the Asuogyaman district of the Eastern region of Ghana.

Figure 3.4: Maize samples in hermetic polyethylene bags before transporting to the pesticide residue laboratory at Shiashie, Accra.

3.5 Sample Extraction

The extraction of the pesticide residues from the maize samples was done using a modified version of Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method at the pesticide residue laboratory of Ghana Standards Authority. The 1 kg samples collected from each town in a region were first put together and mixed thoroughly to give one composite sample per region. For each region, 1 kg composite samples were thoroughly mixed and a FOSS homogenizer 2096 was then used to homogenize the composite maize samples to powdery form. Powdered sample (10 g) was then weighed for each region using a Mettler Toledo PG1003-S mass balance into a 50 ml centrifuge tube. 10 ml each of cold deionised water and acetonitrile were added. The mixture was then vortexed for one minute using a Thermolyne maxi mix plus. A mixture of QuEChERS salts was then added [4 g anhydrous $MgSO_4$ plus 1g NaCl, 1 g Trisodium Citrate Dihydrate (TSCD), and 0.5 g disodium hydrogen citrate sesquihydrate (DHS)]. The mixture was vortexed for a further one minute and then centrifuged for five minutes at 3000 rpm.

3.6 Sample Clean-up and Concentration

Dispersive Solid Phase Extraction (SPE) was the method used in purifying the sample extract. An aliquot (6 mL) of the extract was transferred into a Polypropylene (PP) centrifuge tube containing 150 mg primary and secondary amine (PSA) and 900 mg $MgSO_{4.}$ The mixture was vortexed for one minute and centrifuged for five minutes at 3000 rpm. 4 ml of the supernatant (clean extract) was transferred into a pear-shaped flask. 40 µL of 5% formic acid in acetonitrile (v/v) was then added to adjust the pH. The filtrate was then concentrated to dryness at 35 ^{o}C on rotary evaporator. Ethyl acetate (1 ml) plus 20 µL of 1 % polyethylene glycol was then used to re-dissolve dry concentrate. Sample was then transferred into a 2 mL standard opening auto sampler vials for quantification by GC-ECD for orgacnochlorines and GC-MS for atrazine.

3.7 Sample Analysis for Lindane and other organochlorines using GC-ECD

Samples were analyzed for Lindane and other organochlorines in a gas chromatograph (Varian CP-3800 GC-ECD with CombiPAL autosampler). It was fitted with a Varian analytical column 30 m long + 10 m EZ Guard. The column has a 0.25 mm id fused silica capillary coated with VF-5 ms (0.25 micrometer film). Extracts of samples were interspersed with analytical standards of organochlorines placed on autosampler with standards at the start, between every 10 samples and the last of the GC sample run. The lindane and other organochlorine residue components were identified by comparing their retention times with those of the standards. The GC-ECD conditions are as in table 3.1.

Table 3.1: Chromatographic conditions as set by the pesticides residue laboratory of Ghana Standards Authority.

Apparatus:	
Instruments	**Description**
Gas Chromatograph	Varian CP-3800 GC-ECD with a CombiPAL Autosampler
Analytical Column	30m + 10m EZ Guard x 0.25mm internal diameter fused silica capiliary coated with VF-5ms (0.25µm film) from Varian Inc.
Temperature:	
Item	**Conditions**
Injector	Splitless mode, temperature 270^{0}C
Oven	70^{0}C/ 2min $\underline{25^{\circ}C/min}$ 180^{0}C/Min $\underline{5^{\circ}C/min}$ 300^{0}C
Detector-ECD	300^{0}C
Gases:	
Gas	**Flow rate**
Nitrogen (carrier)	1 ml/min constant flow
Nitrogen (Make-up)	29 ml/min

3.8 Analysis of Atrazine

All samples were analyzed for atrazine in gas chromatograph – mass spectrometer (GC-MS) equipped with 40 m x 0.25 mm x 0.25 µm and VF-5 ms column (phenomenex) (CP–3800/Saturn 2200, Varian, Australia). Extracts of samples were interspersed with analytical standards of atrazine placed on autosampler with standards at the start, between every 10 samples and the last of the GC sample run. The atrazine residue components were identified by comparing their retention times with those of the standard atrazine. Quantification was based on comparison with calibration curves in the concentration range of 0 to 1.0 µg/L. The gas chromatograph – mass spectrometer conditions were as follows: initial temperature was 80 °C and held for 1 minute, then increased to 180 °C at 25 °C/min and increased to 300 °C at 15 °C/min and held for 2 minutes, with total time being 21 minutes. The carrier gas used for the system was Helium and it flowed at a rate of 1.3 ml/min. The injector port was maintained at a temperature of 250 °C. A 10 µl volume was injected in the splitless mode. The ion trap mass spectrometer was operated in the auto-electron impact mode at a voltage of 70 eV. The ion trap, manifold and transfer line temperatures were set to 210 °C, 80 °C and 260 °C respectively. Its mass range is from 40 – 450 m/z.

3.9 Quality Control/Quality Assurance

Quality control and quality assurance as prescribed by the CODEX Alimentarus Committee were incorporated in the analytical scheme. Quality assurance measures applied in the laboratory included rigorous contamination control procedures (strict washing and cleaning procedures), monitoring of blank levels of solvents, equipment and other materials, analysis of procedural blanks, recovery of spiked standards, monitoring of detector response and linearity. During extraction, blanks and duplicates were included in the analysis and re-calibration standards run frequently to check the integrity of the calibration curve. Aliquot (50ml) of each solvent was concentrated to 1 mL and analyzed to check the contamination from the reagents (FAO/WHO, 2007). The quality of analytical methods was assessed by atrazine and organochlorine recovery experiments with maize. Maize matrices that had been assayed and were known to have no detectable levels of the atrazine and organochlorines were used for recovery test. The validation and chemical recoveries were estimated by spiking atrazine-free and lindane-free maize blanks with atrazine and lindane standards overnight before extraction. Samples were extracted and cleaned-up as described above and subsequently analyzed by GC-ECD and GC/MS. The percentage recovery was calculated using the formula:

$$\text{Recovery (\%)} = \frac{\text{Conc. of Pesticide residue recovered from fortified sample}}{\text{Concentration of pesticide added to sample}} \times 100$$

3.10 Preparation of calibration curve

Calibration curves were prepared for atrazine and organochlorines. Atrazine and organochlorine standard solutions of concentrations of 0.1. 0.5 and 1.0 µg/L, were prepared in addition to the blank. An aliquot (10.0 µL) of each concentration was injected into the GC-MS and GC-ECD respectively and the responses recorded. A calibration curve was constructed by plotting the concentration against their respective peak areas.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Introduction

This section presents the field survey as well as the laboratory analysis for discussion. Tables and charts are used to show trends. IBM SPSS statistics 20 together with Microsoft Excel 2007 were used in the analysis of Data obtained from questionnaire.

4.2 Field survey

4.2.1 Demographic Characteristics

Table 4.1 shows the various demographic characteristics of the respondents in the study area. The respondents in the survey included 77 farmers and 34 traders; 76 males and 35 females (Table 4.1).

Table 4.1: Demographic characteristics and level of education of respondents

VARIABLE	FREQUENCY	PERCENTAGE (%)
AGE		
13-19	16	14.4
20-45	35	31.5
46-60	26	23.4
61-Above	34	30.6
GENDER		
Male	76	68.5
Female	35	31.5
EDUCATIONAL STATUS		
Illiterate	31	27.9
Basic	51	45.9
Second Cycle	20	18.0
Tertiary	9	8.1

4.2.2 Respondents’ Usage of Lindane, Atrazine and Pesticides in General

Figure 4.1 shows the responses of farmers and traders to usage of organochlorines and atrazine pesticides in the study area.

All respondents (100%) used pesticides in the maize production process. However, only 18% used Lindane (a possible reason could be the ban status of Lindane being upheld) while 82% used Atrazine (Fig. 4.1).

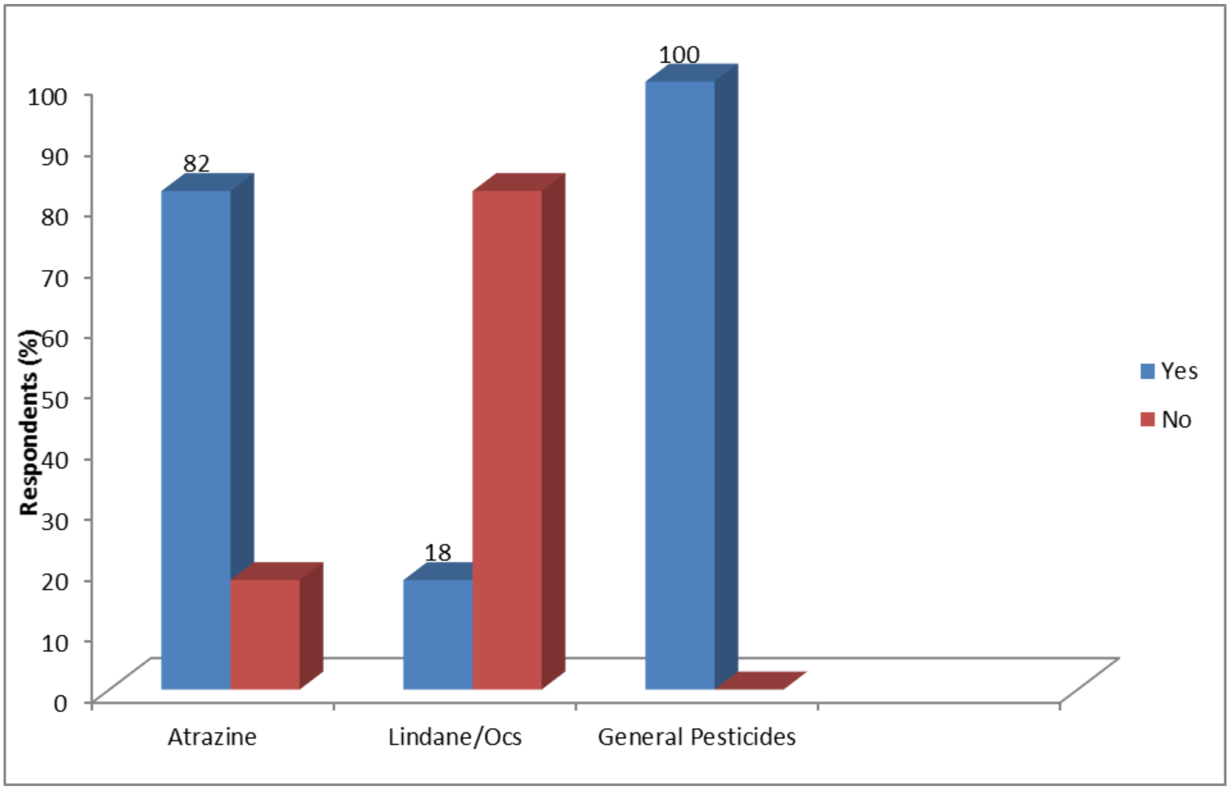


Figure 4.1: Respondents’ usage of Lindane, Atrazine and Pesticides in general.

Respondents (80%) were of the view that they use atrazine in their farming activities since it is as a major herbicide which is easily available and affordable. These pesticides were easily found within the reach of the respondents. Majority (81.6%) got theirs from sales agents who themselves may not be well vexed in safe handling of these pesticides.

Only 8.7% got them from extension officers while 19.7% got them from licensed agrochemical shops (Fig. 4.2).

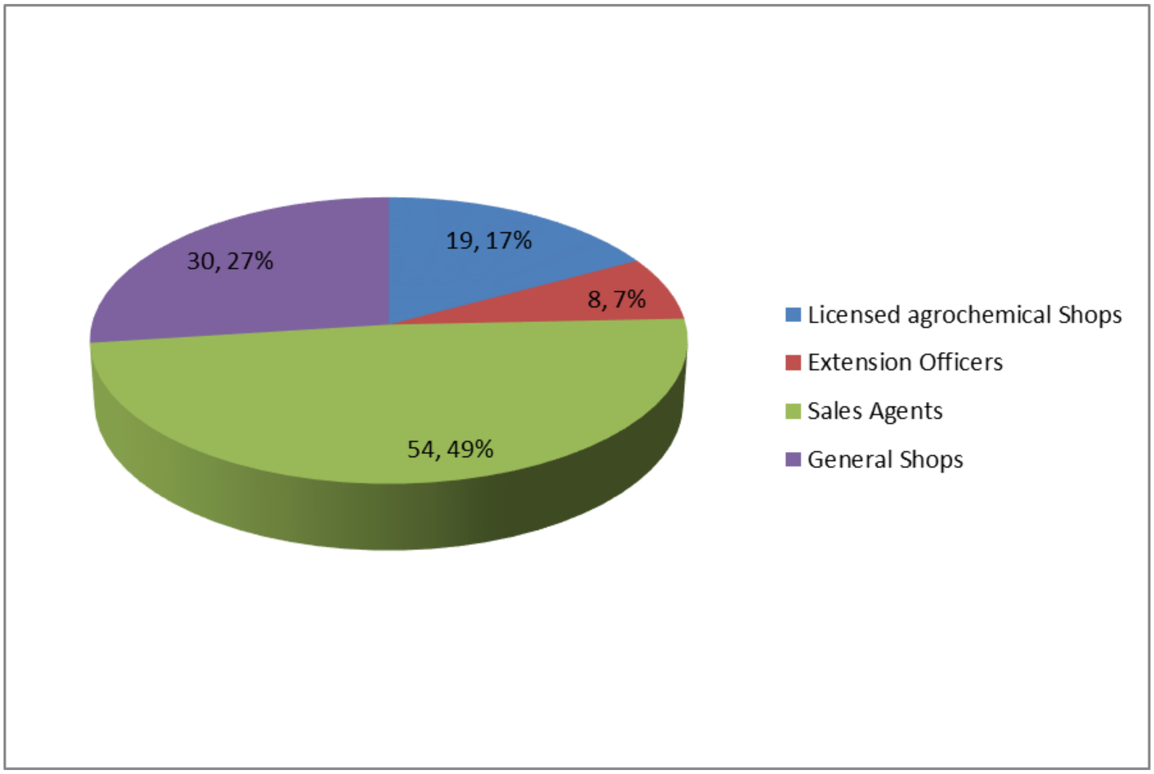


Figure 4.2: Respondents' sources and access to these chemicals

Respondents (83%) indicated that they stored these synthetic chemicals in multipurpose storage structures together with food items and farm implements/tools. This practice is very unsafe as chemicals may contaminate food items and cause poisoning. In 2011 for instance, seven people out of a family of eight died from atrazine poisoning in Shaibuipe in the northern region of Ghana (Der et al, 2013). It was revealed that the family consumed beans contaminated with atrazine. If measures were taken to avert the recurrence of this unfortunate incident, then they are clearly not being enforced, as the practice of storing food items together with agrochemicals is still being practiced.

4.2.3 Practices, Toxicity awareness and Symptoms among respondents

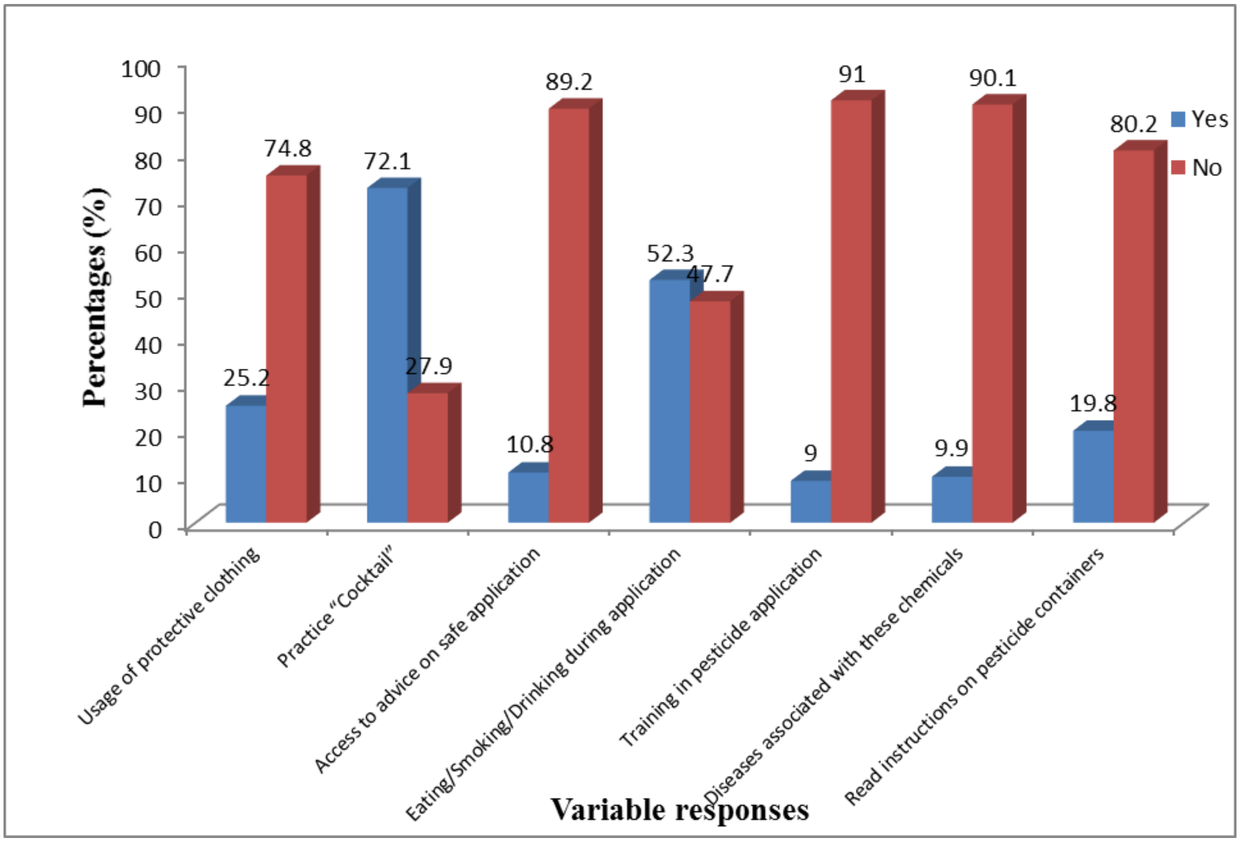


Figure 4.3: Respondents' Practices and toxicity awareness

The survey revealed that 72.1% of the respondents practice "cocktail"; which is a method of applying pesticides in mixtures. Tank mixtures of two or more pesticides were observed in this study. There were no specific instructions for farmers either from extension officers or on the label of these pesticides concerning tank mixtures. These farmers asserted that tank mixing was favourable because it saved time since they could apply more than one pesticide at a time. Moreover, they asserted that it also saved them cost and labour. A farmer in the Lower Manya district of the Eastern region for instance, mixed atrazine and glyphosate for pre-emergence pesticide application. "Cocktailing" is not a safe method of pesticide application because labelled instructions on pesticide containers do not cover mixtures of two or more pesticides and also give no information on the compatibility of active and inert ingredients. In a 2002 study for instance, it was observed that a mixture of fungicides, insecticides and water mineral content influenced the efficacy of individual pesticides against fungal pathogens and insect mortality respectively. Some tank mixtures were also found to induce phytotoxicity on tomato (Smit *et al*., 2002). There is however limited information on the reaction and the effects of tank mixtures observed in this study. In another study, mixtures of insecticides generally resulted in the simultaneous development of resistant strains (Ngowi *et al.*, 2007). Farmers interviewed did not regard "cocktailing" as a less effective practice that could have adverse effects on their health and the environment; rather, "cocktailing" was believed to save time, labour, cost as well as give high pest killing frequency. The observation of various tank mixtures is an indication of the lack of basic knowledge of safe pesticide application by respondents.

Moreover, 74.8% of respondents do not use protective clothing, such as goggles, gloves, long boots, overalls etc. Several reasons ranging from personal discomfort to lack of finances were given though a few wear long-sleeved clothes during the application process. One reason given by respondents for not wearing them is that the warm weather makes the wearing of protective gear impractical due to the possibility of heat stress. The results of a similar study in Egypt showed that 100% of farmers did not use protective clothing. Reasons given were low level of knowledge about the safety measures, unavailability of protective devices at governmental agricultural associations and their high cost at private sector (Gaber and Abdel-Latif, 2012). This finding is also consistent with the results of many other studies conducted in many parts of the world (Mekonnen and Agonafir, 2002; Ngowi, 2003; Ngowi et al., 2007; Williamson et al., 2008; Sneldera et al., 2008; Khan et al., 2009). Hot weather was among the causes of low use of protective clothing in a study conducted in the United States of America (Rucker, 1994). In addition to the above, 52.3% of respondents also eat, drink or smoke during the pesticide application process. Most of the respondents regardless of their level of education practice this hazardous act. Similar behavior was reported in other developing countries (Gomes et al., 1999; Jones et al., 2009). Though 47.7% do not practice this hazardous act, there is the need for education to reduce the numbers that practice this act. Figure 4.3 also shows 91% of respondents do not have any form of training in pesticide mixing and application while 89.2% do not have access to advice on safe application practices from relevant authorities. The fact that majority get their chemical supplies from sales agents who themselves do not have any training in pesticide application may be a contributory factor. In addition to the above, 90.1% of respondents were unaware of the various diseases associated with exposure to these chemicals. In a similar study by Ntow *et al.*, (2006), it was reported that 85% of the respondents do not use protective clothing during pesticide application while 67% of them eat or drink when mixing or applying pesticides. It was also reported in the same study that 91% of the respondents had not received any training whatsoever on pesticides and the use of agrochemicals.

This study also revealed that 80.2% of respondents (Fig. 4.3) do not read instructions on pesticide containers. The main reason given was their inability to read and lack of awareness about any use these instructions could offer them. Farmers saw reading instructions as a time wasting venture. In a similar study in Egypt by Gaber and Abdel-Latif (2012), 67% of applicators did not read instructions on containers. A study conducted in Ethiopia also found most pesticide applicators did not read instructions on pesticides packages due to illiteracy or they are just reluctant to read them (Mekonnen and Agonafir, 2002). Though traders in the study area did not use atrazine, some use other kinds of insecticides such as phosfitab for protection of the maize during storage against insect pests.

4.2.4 Symptoms of pesticide poisoning experienced respondents

Various symptoms were reported by respondents as seen in Fig. 4.4. Headache was the most reported symptom followed by weakness and dizziness. Only 14% (16) of respondents reported no symptoms. In a similar study in Tanzania, Ngowi et al, (2007), reported 31% of respondents with dizziness, 31% with headache and 15% with stomachache. Kishii et al., (1995) reported that farmers assume that pesticide poisoning symptoms are normal and as such they get used to them. In a similar study in Cote d’voire, Ajayi reported that applicators of pesticides tended to accept certain levels of illness as an expected and normal part of the work and thus do not report these symptoms at official health centers for treatment (Ajayi, 2000). The symptoms recorded in this work however are not specific to pesticide exposure and could be due to other causes such as malaria and/or general fatigue.

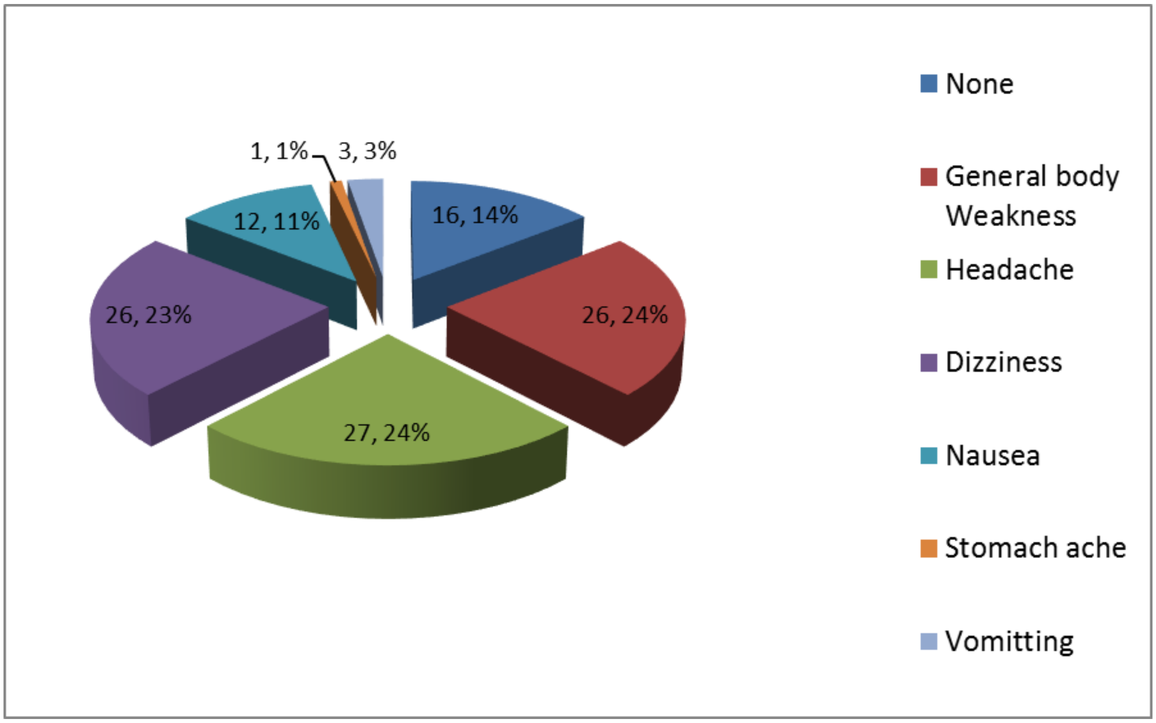


Figure 4.4: Symptoms of pesticide poisoning reported by respondents

4.2.5 Attitude and awareness of farmers towards pesticides hazards

Table 4.2 shows the attitudes of respondents towards adverse impacts of pesticides.

Table 4.2: Attitudes towards adverse impacts of pesticides

	Completely agree Frequency (%)	Agree Frequency (%)	Neutral Frequency (%)	Disagree Frequency (%)
Pesticide use could lead to Ground/Surface water pollution	2 (1.8%)	24 (21.6%)	73 (65.8%)	12 (10.8%)
Use of pesticides causes air Pollution by toxic compounds	30 (27.0%)	67 (60.4%)	14 (12.6%)	None
Pesticides contribute to pollution and reduce soil fertility	3 (2.7%)	29 (26.1%)	72 (64.9%)	7 (6.3%)
Pesticides contribute to elimination of beneficial organisms in soil and nature	3 (2.7%)	30 (27.0%)	73 (65.8%)	5 (4.5%)
Wrong use of pesticides could lead to human death	3 (2.7%)	80 (72.1%)	27 (24.3%)	1 (0.9%)
Applying pesticides to maize increases yield	9 (8.1%)	67 (60.4%)	33 (29.7%)	2 (1.8%)

From Table 4.2, respondents generally were less aware of the potential adverse impacts of pesticide deposition on the environment. Though 68.5% believe that pesticides application increases maize yield and over 74% believing that wrong use of pesticides could lead to human death, a significant amount (68.5%) were neutral about the notion that Pesticides contribute to elimination of beneficial organisms in soil and nature. These respondents are most likely unaware of the negative effects of these chemicals on biodiversity (bees, birds and soil ecosystem). Moreover, 68.5% are also neutral about the notion that pesticide use could lead to Ground/Surface water pollution while 64.9% were neutral about the notion that Pesticides contribute to pollution and reduce soil fertility. However, 67.4% believe that the use of pesticides causes air pollution by toxic compounds. This probably may be because they smell these chemicals during application as most do not use protective clothing. Though a study by Andersch and Anderson in 1991 showed that 31 different pesticides when applied at recommended doses had no long term influence on soil nitrogen mineralization, absence of national regulations on sustainable use might lead to detectable concentrations in ground and surface water (Andersch and Anderson, 1991).

4.3 Levels and distribution of atrazine, Lindane and other Organochlorines in maize

The overall mean recoveries from fortified samples were between 80% and 120 %. Recoveries for this study were within the normal acceptable range of 65 – 120% (Hill, 2000; FAO/WHO, 2003; FAO/WHO, 2007).

A total of 9 composite samples representing 9 regions were analysed. Results are presented in Table 4.3 for Ashanti region (AR), Brong Ahafo region (BAR), Eastern region (ER), Northern region (NR), Central region (CR), Upper east region (UER), Upper west region (UWR), Volta region (VR) and Western region (WR). The maximum residue levels (MRLs) of these pesticides in maize for the European Union are shown in table 4.3.

Table 4.3: Levels of atrazine, lindane and other organochlorines in composite maize samples (mg/kg)

	AR	**BAR**	**ER**	**NR**	**CR**	**UER**	**UWR**	**VR**	**WR**	**EU MRLs for Maize**
Lindane	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.01(Max)
Endrin	< 0.005	0.005-0.007	< 0.005	< 0.005	0.005-0.006	< 0.005	< 0.005	< 0.005	< 0.005	0.01(Max)
Heptachlor	< 0.005	0.005-0.027	0.005-0.020	< 0.005	0.005-0.021	< 0.005	0.005-0.023	< 0.005	< 0.005	0.01(Max)
β-Endosulfan	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.10(Max)
Dieldrin	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.01(Max)
P’P-DDE	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.05(Max)
P’P-DDT	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.05(Max)
P’P-DDD	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.05(Max)
ɣ -Chlordane	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.01(Max)
Methoxychlor	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.01(Max)
Delta-HCH	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.01(Max)
β-HCH	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.01(Max)
Endosulfan sulfate	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.10(Max)
Alpha- Endosulfan	< 0.005	< 0.005	0.005-0.013	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.10(Max)
Aldrin	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.01(Max)
Atrazine	0.010-0.05	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.10(Max)

The highest level of atrazine (0.05 mg/kg) was recorded in the Ashanti region (AR) while lindane and the other OCPs were below the detection limit of 0.005 mg/kg in this region. Atrazine in all the other 8 regional composite samples were below the limit of detection (0.010 mg/kg). Though lindane was below detection limit for all samples, some amount of other organochlorines pesticides were detected in some samples. Endrin was found in central (0.006 mg/kg) and Brong Ahafo (0.007 mg/kg) regions while heptachlor was found in Brong Ahafo (0.027 mg/kg), Eastern (0.020 mg/kg), Central (0.021 mg/kg) and Upper West (0.023 mg/kg) regions. Alpha endosulfan (0.013 mg/kg) was also found in maize samples from the Eastern region. From the data (Table 4.3), concentrations of atrazine in majority of the samples were below detection limit. The concentration measured for Ashanti region was also low (0.05 mg/kg). These relatively low levels of Atrazine may be due to soil characteristics as well as chemical and biological breakdown of Atrazine. Its persistence is also dependent on soil characteristics such as pH, organic matter content, moisture and texture (Kerle et al., 2007). From the soil fertility data in table 4.4, the relatively higher pH of Ashanti soils might be responsible for the measured levels of atrazine found in maize samples in this region. Atrazine is a slightly basic herbicide and as such is easily absorbed in slightly acidic soils but persistent in high pH soils (Phewnil et al., 2010; Hiltbold et al., 1997). At the relatively low pH values in the other regions, chemical and biological breakdown occur faster; degrading Atrazine from the environment (Hager et al., 2008).

Table 4.4: Average soil fertility status of some regions

Region	Soil pH	Organic matter (%)	Total nitrogen (%)	Available P (mg/kg soil)	Available Ca (mg/kg soil)
Ashanti	4.3 - 7.8	1.5 - 3.0	0.1 - 0.3	0.1 - 12.0	50 – 100
Brong Ahafo	3.5 - 6.7	0.3 - 1.7	N/A	0.1 - 64.3	16 – 140
Northern	4.5 - 6.7	0.6 - 2.0	0.02 - 0.05	2.5 - 10.0	45 – 90
Upper East	5.1 - 6.8	1.1 - 2.5	0.06 - 0.14	1.8 - 14.8	44 - 152
Upper West	6.0 - 6.8	0.5 - 1.3	0.01 - 0.07	2.0 - 7.4	52 - 152
Western	3.8 - 7.1	1.0 - 5.7	0.06 - 5.4	0.4 - 11.3	28 - 420

Source: Soil Research Institute (SRI) CSIR - Kumasi.

Lindane being below detection limit for all samples could be due to the ban on this pesticide. Lindane use has been banned in Ghana since 2009 and its popularity and use has dwindled ever since. From Fig. 4.1only 18% of respondents use Lindane and other organochlorines. Though lindane was below detection, endrin (0.007 mg/kg for Brong Ahafo region; 0.006 mg/kg for central region), and heptachlor (0.027 mg/kg for Brong Ahafo; 0.020 mg/kg for Eastern region; 0.021 mg/kg for central region; 0.023 mg/kg for upper west region) exceed the MRL of 0.01 mg/kg set by the European Union for maize. Alpha-endosulfan (0.013 mg/kg) however is almost same as the EU-MRL for maize. Though the production and use of these chemicals (endrin, endosulfan and heptachlor) have been discontinued, their persistence in the environment as a result of their stability may be responsible for their presence in the samples from the Brong Ahafo, Eastern, Central and Upper West regions. Heptachlor for instance was used from 1953 to 1974 as a soil and seed treatment to protect corn, small grains, and sorghum from pests (ATSDR, 2007).

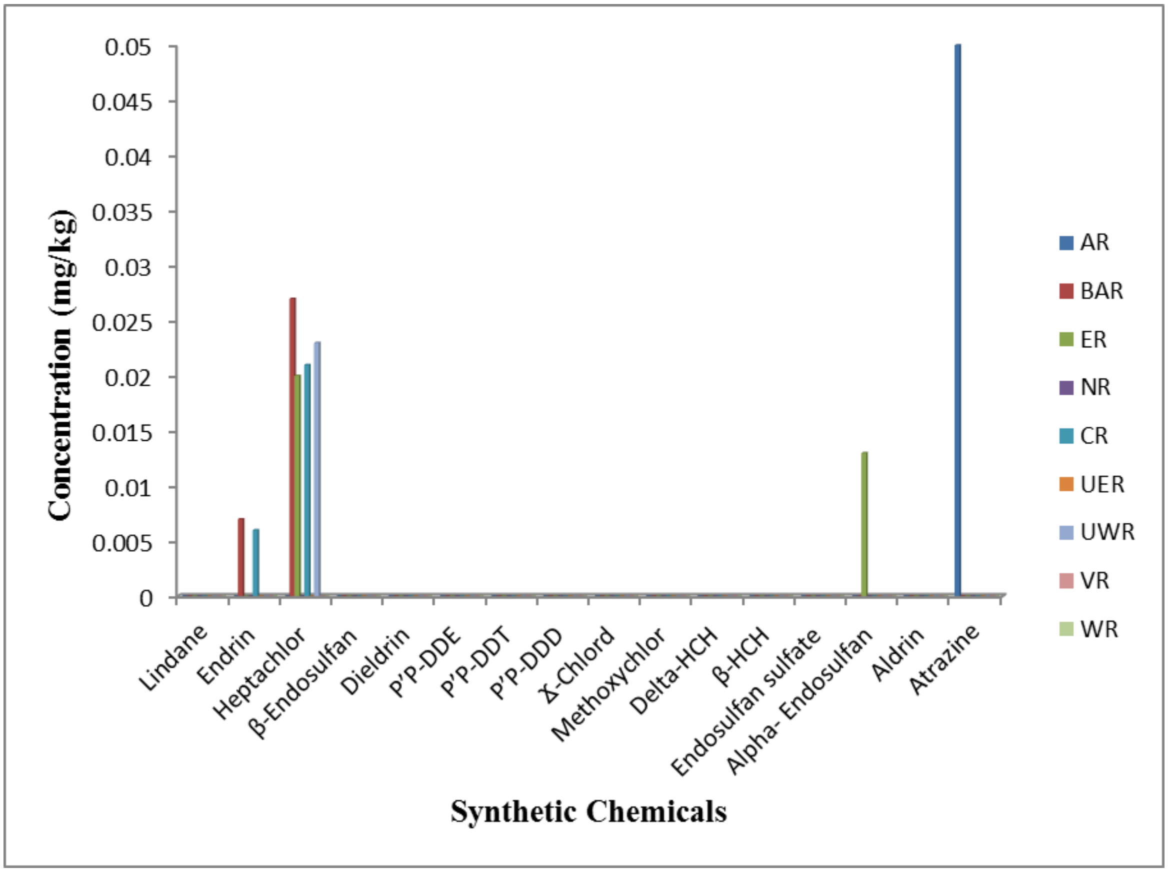


Figure 4.5: levels of Pesticides in the different sampling regions

4.4 Health Risk Estimation

To assess the risk of pesticide usage to farmers and consumers, the guidelines for potential risk assessment drawn up by the US EPA were followed (US EPA, 1998). A reference dose (RfD) was used to evaluate the chronic risk posed by exposure to these pesticides. It is the level at or below which daily aggregate exposure to pesticides over a lifetime will not cause any significant risk to human health. It thus serves as a point of reference from which the potential health effects of these pesticides at other doses could be estimated. It is derived from the 'no observable adverse effect levels' (NOAEL) of the chemicals and exposures at or below the RfD are considered acceptable by the USEPA (USEPA, 2002a).

In this study, health risk estimates were calculated by integrating the pesticide analysis data from the study and the consumption rate of food (maize) in Ghana.

The following assumptions were adopted from the U.S Environmental Protection Agency's guidelines:

(a). Hypothetical body weight of 10 kg for children (0-1yrs), 30 kg for children (1-11yrs) and 70 kg for adults.

(b). Maximum absorption rate of 100% and a bioavailability rate of 100% (USEPA, 1998)

(c). Maize consumption rate in Ghana is 8.48 kg/day (FAO, 2012).

Consumption of contaminants in maize was calculated based on its concentration in the food and on an estimate of the food consumption rates. Hence for each type of exposure, the lifetime exposure dose (mg/kg/day) was obtained by multiplying the residual pesticide concentration (mg/kg) in the food of interest by the food consumption rate in the country (kg/day) and dividing the product by the body weight (kg) (US EPA, 1989; Tchounwou *et al.*, 2002). The hazard indices for children and adults were estimated as ratios between estimated pesticide exposure doses and the reference doses (RfD).

Pesticide residue data from maize samples (Table 4.3) were used for the health risk assessment. It is important to note that the data used represent the maximum concentrations of pesticide residues in the maize samples. Since a conservative approach is needed in dealing with risk assessment of multiple chemical compounds, it was more appropriate to consider the maximum levels of specific detected pesticides instead of their mean concentrations (Tchounwou *et al*, 2002). A hazard index (HI) <1 is considered to have an unlikely adverse health effect, while HI > 1 is said to have probable adverse health effect on consumers.

$$\text{life exposure dose} = \frac{\{\text{Residue conc.in maize}\} \times \{\text{Maize (food)consumption rate}\}}{\{\text{Body Weight}\}} \quad \text{---------} \ 1$$

$$\text{Hazard Index (HI)} = \frac{\text{Exposure(Estimated) dose}}{\text{Reference dose (RfD)}} \quad \text{--------} \ 2$$

Table 4.5 represents the estimated dose values and health hazards associated with synthetic chemical residues in maize from the study area. Hazard indices were computed for children within the age ranges of 0 – 1year, 1 – 11years and adults for three organochlorines (endrin, alpha-endosulfan and heptachlor for Brong Ahafo, Eastern, Central and Upper West regions) as well as atrazine (for Ashanti region). Data analysis of health risk estimates indicated that all these chemicals do not pose a direct hazard to human health, although they are present in maize samples from the study area. Their potential for systemic toxicity in all age groups is thus low. The hazard indices for these chemicals (table 4.5) also indicate an unlikely adverse health effect since the estimated doses exceeded the recommended reference doses for all the chemicals.

Table 4.5: **Estimated dose values and Hazard indices of organochlorine and atrazine residue exposure through maize consumption in the study area.**

Chemicals (Region)	RfD	Estimated Dose (mg/kg/day)			Hazard Index (HI)		
		0-1yr	1-11yr	Adult	0-1yr	1-11yr	Adult
Endrin(Brong Ahafo region)	0.20	5.94×10^{-3}	1.98×10^{-3}	8.48×10^{-4}	0.030	0.010	0.004
Endrin(Central region)	0.20	5.09×10^{-3}	1.70×10^{-3}	7.27×10^{-4}	0.026	0.009	0.004
Heptachlor (Brong Ahafo region)	0.10	0.0229	0.0076	0.0033	0.229	0.076	0.033
Heptachlor(Eastern region)	0.10	0.0170	0.0057	0.0024	0.170	0.057	0.024
Heptachlor (Central region)	0.10	0.0178	0.0059	0.0025	0.178	0.059	0.025
Heptachlor (Upper west region)	0.10	0.0195	6.50×10^{-3}	2.79×10^{-3}	0.195	0.065	0.028
Alpha-endosulfan (Eastern)	0.05	0.0110	3.67×10^{-3}	1.57×10^{-3}	0.220	0.073	0.031
Atrazine (Ashanti region)	0.10	0.0424	0.0141	0.0061	0.424	0.141	0.061

Although residue levels of the organochlorines and atrazine were below the maximum permissible intake of Codex Committee on pesticides residues (Sun *et al.*, 2006), as well as EU-MRLs (http://secure.pesticides.gov), there was no zero risk because pesticides were present in the maize samples though they are below detection limit in some cases. Since chemicals are generally persistent, volatile, lipophilic and bio-accumulative both in the environment and at each trophic level of the food chain, they have the potential to reach high concentrations through bio-magnification in the tissues of predators including humans, which are high on the food chain (Kishimba, 2004). This can lead to adverse environmental and human health effects.

These outcomes may be due to the fact that organochlorines pesticides lindane, endrin, heptachlor, β-endosulfan, dieldrin, p'p-DDE, p'p-DDT, p'p-DDD, gamma-Chlordane, methoxychlor, delta-HCH, β-HCH, endosulfan sulfate, alpha- endosulfan and aldrin have been banned from use in Ghana. However the little residues may be due to their persistence and stability in the environment. Atrazine levels in the Ashanti region may also be due to the fact that Atrazine is a slightly basic herbicide and as such is easily absorbed in slightly acidic soils but persistent in high pH soils (Phewnil et al., 2010; Hiltbold et al., 1997). At the relatively low pH values in the other regions, chemical and biological breakdown occur faster; degrading Atrazine from the environment (Hager et al., 2008). In the Ashanti region, however, the higher pH values may have led to the persistence of atrazine in the environment hence its presence in small quantities in maize samples from this region.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

It is clearly seen from the results of the study that farmers and traders in the Brong Ahafo, Eastern, Ashanti, Central, Northern, Upper West, Volta, Upper East and Western regions of Ghana do not follow appropriate safety precautions during pesticide application. Applicators thus experience clinicopathological conditions including nausea, vomiting, abdominal cramps, itching, stomach-ache, dizziness and headache. Though lindane was below detection level in the study area, a detectable level of atrazine was found in Ashanti region. Detectable levels of other organochlorines (endrin, heptachlor and alpha-endosulfan) were found in maize from Brong Ahafo, Eastern, Central and Upper West regions.

The estimated dose for all the organochlorines and atrazine do not pose a direct hazard to human health although they are present in the maize samples since their values were lower than toxic thresholds as well as reference doses. Residue levels of these pesticides are below the maximum permissible intake, but there was no zero risk because pesticides were present in maize samples though below detection limit in some regions. Thus due to the unsafe application practices by applicators, there exists a potential risk for systemic and carcinogenic health effects by these chemicals in the study area.

5.2 Recommendations

- The environmental protection agency (EPA) must ensure the establishment of effective protective measures, integrated pest management practices, organic farming and reducing the volume of synthetic organic chemical usage as the focus of risk prevention.

- A comprehensive study to assess the inherent weaknesses in the health education programme regarding the safe use of pesticides and other synthetic organic chemicals in maize production should be conducted by the EPA and Ministry of food and agriculture (MOFA)
- A policy should be made by the EPA to halt the practice where sales agents of these chemicals are increasingly becoming the source of advice to farmers on pesticide use. These agents must be forced by the policy to refer farmers and traders to extension officers for advice.
- Good agricultural practices must be encouraged by MOFA
- The Chemicals control and management directorate of the EPA must make a policy to establish a pesticide management fund into which pesticides sale and marketing companies would be directed to make contributions to support pesticide monitoring activities by the EPA.
- Similar studies should be conducted to ascertain pesticide level and distribution at the district level to enable effective implementation of safe practice training programmes by the government and NGOs

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APPENDIX

APPENDIX I

SCHOOL OF NUCLEAR AND ALLIED SCIENCES, UNIVERSITY OF GHANA

Questionnaire to find out farmers' and traders' knowledge, practice and attitudes regarding safe use of Atrazine and Lindane as well as toxicity awareness and symptoms among farmers and traders who use these chemicals.

(Please either fill in the blank space provided or tick where applicable)

Socio-Demographic Profile

1. Respondent Name ________________________________
2. District ____________________ Village_______________ Region ________________
3. Age 13-19 ☐
   20-45 ☐ 46-60 ☐
   61-above ☐
4. Gender: Male ☐ Female ☐

5. Occupation…………………

6. Level of education Basic☐ Second cycle ☐ Tertiary ☐ Illiterate ☐

<u>Information Sources and Knowledge on Pesticide use and Management</u>

7. Where did you first hear about the Pesticide you are using?

____ 1) Neighbor/other farmers

____ 2) Extension officer

____ 3) Pesticide sales agents

____ 4) Mass media

_____ print

_____ billboard

_____ radio

____ 5) other (please specify) ___________________

8. What is your <u>most important</u> consideration in deciding what Pesticide to buy?

_____ 1) Price

_____ 2) Effectiveness (killing efficiency)

_____ 3) Packaging

_____ 4) Endorsement by sales agent

9. Do you use pesticides on your maize Yes ☐No ☐

10. If Yes what is the purpose of using the pesticides………………

11. What time of the day do you normally apply the pesticides?

Morning ☐ Afternoon ☐ Evening ☐

12. By what medium did you learn about how to apply the pesticides?

Air/Radio/TV ☐ Instruction on container ☐Extension officer ☐ fellow

Farmers☐ Others ☐

Practices

13. Do you have any training in pesticides application Yes☐ No ☐

14. If Yes, Who taught you…………………

15. Do you read the instructions on the pesticide containers Yes☐ No ☐

16. Do you reuse the pesticide container or you dispose it off completely. Use☐ Dispose ☐

17. Where did you get your pesticides from ……………

18. Do you practice cocktail of pesticides Yes ☐ NO☐ and Why…………..

19. Do you put on protective clothing when applying pesticides Yes ☐No ☐

20. If Yes which one …………

21. Is there any extension/ veterinary officer around who always advises you as to how to use the pesticides safely? ☐ Yes ☐ No

22. Do you eat/drink/smoke when applying pesticides? Yes ☐ No ☐

23. Have you ever had any training in pesticides applications before Yes ☐ No ☐

Toxicity Awareness and Symptoms of Lindane and Atrazine

24. Have you ever heard of Atrazine? Yes ☐ No ☐

If yes, what is it…………......

25. Have you heard of Lindane? Yes ☐ No ☐

If yes, what is it? ………………..

26. Do you know whether Atrazine can cause any human health problems? Yes☐ No ☐

27. Do you know whether Lindane can cause any human health problems? Yes ☐ No ☐

28. Have you ever personally experience some sickness before as a result of the use of Lindane

Yes ☐ No ☐

29. If yes, Outline two of the symptoms……………..

30. Have you ever personally experience some sickness before as a result of the use Atrazine

Yes ☐ No ☐

31. If yes, Outline two of the symptoms……………..

32. Do you know some of the diseases that are associated with Atrazine usage Yes No

33. If yes state one health problem………

34. Do you know some of the diseases that are associated with Lindane usage Yes No

35. If yes state one health problem………

<u>Attitudes toward Pesticide Use</u>

Please indicate whether you completely agree, agree are neutral, disagree or completely disagree with the following statements:

36. Applying pesticides to maize crop will make the yield go up:

____ 1) Completely Agree

____ 2) Agree

____ 3) Neutral

____ 4) Disagree

____ 5) Completely disagree

37. Wrong use of pesticides could lead to human death

____ 1) Completely Agree

____ 2) Agree

_____ 3) Neutral

_____ 4) Disagree

_____ 5) Completely disagree

38. Use of pesticides contributes to the elimination of beneficial organisms in the soil and nature

_____ 1) Completely Agree

_____ 2) Agree

_____ 3) Neutral

_____ 4) Disagree

_____ 5) Completely disagree

39. Pesticides contribute to pollution and reduce soil fertility

_____ 1) Completely Agree

_____ 2) Agree

_____ 3) Neutral

_____ 4) Disagree

_____ 5) Completely disagree

40. Use of pesticides causes air pollution by toxic compounds

_____ 1) Completely Agree

_____ 2) Agree

_____ 3) Neutral

_____ 4) Disagree

_____ 5) Completely disagree

41. Use of pesticides leads to groundwater/Surface water pollution

_____ 1) Completely Agree

_____ 2) Agree

_____ 3) Neutral

_____ 4) Disagree

_____ 5) Completely disagree